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## CONTENTS

PAGE

Seasonal Variation in the Blood and Bone Marrow of Scottish Hill Sheep. By C. S. Grunsell .. .. .. .. 93

Malignant Neoplasia of the Oesophagus and Rumen of Cattle in Kenya. By W. Plowright .. .. .. .. 108

Melanotic Tumours of Dogs. By E. Cotchin .. .. .. 115

Studies on Ethidium Bromide. VI. The Prophylactic Properties of the Drug. By T. M. Leach, A. A. Karib, E. J. H. Ford and E. C. Wilmshurst .. .. .. .. .. 130

Studies on *Trichostrongylus axei*. III. The Course of Untreated Infestations. By T. E. Gibson .. .. .. .. 143

Studies on Host Resistance to Dictyocaulus Infection. The Phenomenon of Inhibited Development. By J. F. Michel 149

Primary Pulmonary Carcinomas of the Dog. By A. G. Ferri and Eva Tausk .. .. .. .. .. .. .. 159

Immunisation against Contagious Bovine Pleuropneumonia, with Special Reference to the Use of a Dried Vaccine. By F. W. Priestley .. .. .. .. .. .. 168

*Pasteurella haemolytica* Septicaemia of Lambs. By J. T. Stamp, J. A. A. Watt and J. R. Thomlinson .. .. .. 183

# SEASONAL VARIATION IN THE BLOOD AND BONE MARROW OF SCOTTISH HILL SHEEP

By

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## INTRODUCTION

Holman (1944) has shown that a significant decrease in haemoglobin may occur in Scottish hill sheep in late winter and early spring. He considered that this fall was probably due to inanition as he found that with the flush of early spring grass there was a rise in haemoglobin. However, Hawkins and de Frietas (1947) in America, have shown that the fluctuations in the worm egg count during a five year period in sheep at pasture were accompanied by inverse changes in erythrocyte count and haemoglobin level. It would appear possible, therefore, that the changes in haemoglobin levels observed by Holman may have been partly attributable to changes in worm burden, more especially since Morgan and his co-workers have shown a characteristic spring rise in worm egg count in Scottish hill sheep (Morgan and Sloan, 1947; Morgan, Parnell and Rayski, 1950.) In 1949 and 1950, these same workers extended their previous observations on the fluctuations in worm egg count to include a study of the seasonal variations in worm burden in Scottish hill sheep, (Morgan, Parnell and Rayski, 1951).

The work described here is an investigation of the blood picture of the sheep on one of the hefts used. To obtain a better appreciation of the nature of any variations found in the peripheral blood, marrow spreads were also examined in a proportion of the sheep. The results of the estimation of helminth burden (Morgan, *et. al.*, 1951) were made available by Morgan and his co-workers for comparison with the blood and marrow findings. As difficulty was anticipated in separating the possible effects of worm burden from those of the low nutritional plane, which has been shown to exist in Scottish hill sheep in the spring (Orr and Fraser, 1932), it was decided to study the blood and bone marrow in a group of sheep on a diet providing half maintenance, when the worm burden was reduced to negligible proportions.

## TECHNIQUES

*Blood.* Collection was made from the jugular vein into oxalated bottles, (Wintrobe, 1946). The packed cell volume (P.C.V.) was estimated by centrifuging in Wintrobe tubes at 3,000 revolutions per minute for an initial period of fifty-five minutes followed by further periods of five minutes until two identical readings were

obtained. Haemoglobin estimation (Hb) was by Sahli haemoglobinometer, (Holman, 1950). For erythrocyte and leucocyte counts (R.B.C. and W.B.C.) the methods described by Holman (1950) were followed. The mean corpuscular volume (M.C.V.) and mean corpuscular haemoglobin concentration (M.C.H.C.) were calculated according to Wintrobe (1946). Differential leucocyte counts (D.L.C.) were from the examination of at least 200 cells in films stained by Wright's stain, (Coffin, 1945). Films were examined for evidence of regeneration by the inspection of at least 10,000 erythrocytes.

*Marrow.* The technique used for the bioptic sampling of marrow, the preparation and staining of spreads and differential cell counting, has been described, (Grunsell, 1951). The 2nd to the 6th sternebrae were used for puncture where successive samples were collected from the same sheep. Myeloid erythroid (M/E) ratios and maturation curves (m.c.) were calculated from the haemomyelograms. The incidence of mitotic activity was recorded during the differential count. The quantitative measurements of marrow activity were obtained from a grading of the cellularity of the spreads, (Grunsell, 1955) and by total nucleated cell counts of marrow blood, (Whitby and Britton, 1942).

## RESULTS

### *The Blood and Bone Marrow Picture of Sheep on a Diet Providing Half Maintenance*

Six castrated, male, grey-face sheep aged ten months were divided into two groups and numbered E 98, E 99 and E 100 (Group E) and B 198, B199 and B 200 (Group B) respectively. The following measures were adopted to control helminth infestation. In the week before sampling started both groups were given 20 g. of Phenothiazine and reinfestation prevented by box feeding and by the removal of excreta every three days. Faeces worm egg counts using the Gordon-Whitlock technique (Gordon and Whitlock, 1939), were determined before, halfway through and at the end of the experiment and are shown in Table 1. Measured diets provided

Table 1

Worm Egg Counts

| *Sheep No.* | *Worm eggs per g. of faeces collected* | | |
|---|---|---|---|
| | *Before dosing* | *During 4th week* | *At final sampling* |
| E.98 | 2,000 | 200 | 0 |
| E.99 | 1,400 | 400 | 0 |
| E.100 | 7,100 | 900 | 0 |
| B.198 | 1,500 | 900 | 0 |
| B.199 | 5,100 | 0 | 0 |
| B.200 | 2,000 | 0 | 0 |

full maintenance in the case of Group E and half maintenance in Group B. Water was allowed to both Groups ad lib. Blood and bone marrow were sampled on the day before controlled feeding started and thereafter at weekly intervals for six weeks. The sheep were weighed at the time of each sampling. The standards devised by Holman (1944) for the Maximum Allowable Deviation were used to assess the significance of any variation in the successive blood pictures available for each sheep.

Table 2 shows the weekly weights and blood and bone marrow findings for the two groups of sheep on different levels of nutrition.

TABLE 2

WEEKLY WEIGHTS AND BLOOD AND BONE MARROW FINDINGS
Two groups of sheep on different levels of nutrition

| | Sheep No. | Day 0 | 7 | 14 | 21 | 28 | 35 | 42 |
|---|---|---|---|---|---|---|---|---|
| Weight in lb. | E 98 | 108 | 107 | 108 | 104 | 107 | 107 | 108 |
| | E 99 | 91 | 92 | 91 | 90 | 92 | 91 | 92 |
| | E100 | 109 | 110 | 109 | 109 | 103 | 105 | 106 |
| | B198 | 101 | 96 | 97 | 91 | 91 | 89 | 85 |
| | B199 | 111 | 108 | 106 | 102 | 102 | 101 | 101 |
| | B200* | 104 | 102 | 100 | 95 | 94 | 91 | 87 |
| Marrow Grade of Cellularity | E 98 | IV | III | IV | IV | I | II | IV |
| | E 99 | IV | IV | IV | IV | II | III | II |
| | E100 | IV | IV | III | IV | III | II | I |
| | B198 | IV | II | II | III | III | I | I |
| | B199 | IV | III | III | III | III | III | II |
| M/E Ratio | E 98 | 0.79:1 | 0.54:1 | 0.75:1 | 0.70:1 | 2.1 :1 | 1.2 :1 | 0.72:1 |
| | E 99 | 0.50:1 | 0.37:1 | 1.13:1 | 1.40:1 | 1.60:1 | 2.50:1 | 1.30:1 |
| | E100 | 0.66:1 | 0.64:1 | 0.87:1 | 0.32:1 | 0.83:1 | 2.50:1 | 0.89:1 |
| | B198 | 0.52:1 | 0.80:1 | 0.96:1 | 1.70:1 | 1.20:1 | 1.70:1 | 0.86:1 |
| | B199 | 0.51:1 | 0.79:1 | 1.49:1 | 1.50:1 | 1.10:1 | 0.90:1 | 1.20:1 |
| T.M.C. $10^3$/cu.mm. | E 98 | 257 | 122 | 144 | 51 | 39 | 129 | 101 |
| | E 99 | 266 | C | 127 | 117 | 37 | 10 | 48 |
| | E100 | 210 | 121 | 37 | 154 | 145 | C | C |
| | B198 | 195 | 52 | 31 | 45 | 55 | 22 | 22 |
| | B199 | 106 | 25 | 25 | 46 | 59 | 32 | C |
| Peripheral Blood R.B.C. $10^6$/cu.mm. | E 98 | 12.4 | 11.6 | 13.7 | 10.6 | 13.4 | 12.7 | 12.5 |
| | E 99 | 11.5 | 12.9 | 14.2 | 12.6 | 15.2 | 13.7 | 13.6 |
| | E100 | 10.4 | 10.1 | 12.2 | 14.6 | 12.1 | 12.6 | 13.6 |
| | B198 | 11.4 | 12.5 | 13.2 | 13.6 | 13.1 | 12.2 | 12.8 |
| | B199 | 13.2 | 14.1 | 13.1 | 13.0 | 13.1 | 12.9 | 10.6 |
| W.B.C. $10^3$/cu.mm. | E 98 | 11.8 | 17.7 | 16.4 | 12.3 | 17.8 | 17.5 | 13.2 |
| | E 99 | 9.3 | 9.4 | 8.0 | 9.7 | 9.5 | 7.5 | 10.2 |
| | E100 | 9.4 | 10.9 | 11.7 | 10.0 | 13.7 | 12.3 | 11.0 |
| | B198 | 9.8 | 9.4 | 8.8 | 8.8 | 8.7 | 8.6 | 7.9 |
| | B199 | 9.3 | 9.1 | 11.1 | 8.6 | 9.6 | 10.0 | 9.7 |

* B200 developed an infective condition in the third week of the experiment, and therefore measurements for this sheep have been omitted.

C = Sample clotted.

Group B, on a diet providing half maintenance, showed a progressive weight loss which represented, for the group, a loss of approximately 14 per cent over the six weeks, while in Group E which had full maintenance the loss was 0.96 per cent.

Comparison of the results for the two groups showed no effect due to the different planes of nutrition in either blood or bone marrow pictures. There was, however, a significant rise in R.B.C. at the end of the fourth week in E 99 and E 100 and at the end of the third week in B 198. Certain marrow changes which were also common to both groups are illustrated in Fig. 1 which shows the

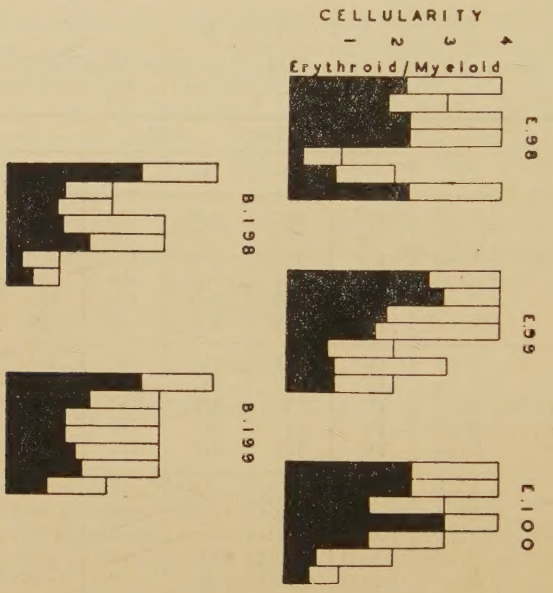


Fig. 1. The marrow picture at weekly intervals for six weeks, of sheep on two planes of nutrition.

a. Diet providing full maintenance —E.98, E.99, E.100.
b. „ „ half „ —B.198, B.199.

M/E ratio related to the grade of cellularity. A reduction in cellularity, largely at the expense of the erythroblastic tissue, occurred in all the sheep with the exception of E 98, during the six weeks covered by the observations. The total nucleated marrow cell counts shown in Table 2 also indicate a reduction in marrow activity. These marrow changes were not reflected in the peripheral blood.

*The Blood and Bone Marrow of Sheep at Pasture*

The observations were carried out in two successive years extending from January to July 1949 and from December 1949 to June 1950 respectively. The sheep were Cheviots and came from

a Scottish Border Hill farm. They had received no anthelmintic treatment during the previous twelve months. These sheep formed the material for a collateral study of the fluctuations in worm burden by Morgan, *et al.* (1951) and were slaughtered for assessment of worm infestation within twenty-four hours of the collection of the samples for haemocytological examination. The worm burden figures were made available to the writer by Morgan and his co-workers.

During the period from January to July 1949 the blood picture of one hundred and ninety-four sheep was examined. They varied in age from a few weeks to seven years old and comprised the population of one heft. The sheep were divided into four groups B, C, D and R and were sampled and slaughtered on January 1st, April 5th, June 7th and July 12th respectively. As shown in Table 3 the

TABLE 3

NUMBERS AND AGES OF THE SHEEP AND THE DATES OF SAMPLING

| | | | *Age of sheep to nearest year* | | | | | | | *Total* |
|---|---|---|---|---|---|---|---|---|---|---|
| *Group* | *Date of sampling* | *Lambs* | 1 | 2 | 3 | 4 | 5 | 6 | 7 | *in Group* |
| B | 11-1-49 | — | 10 | 5 | 6 | 5 | 5 | 5 | 8 | 44 |
| C | 5-4-49 | — | 10 | 5 | 8 | 7 | 6 | 4 | 9 | 49 |
| D | 7–6–49 | 14 | 10 | 5 | 8 | 8 | 6 | 5 | 8 | 64 |
| R | 12-7-49 | 20 | 6 | 2 | 2 | 2 | 2 | 1 | 2 | 37 |

distribution according to age was approximately the same in Groups B, C and D but Group D contained in addition lambs born in mid-April. Group R consisted of twenty lambs and a number of sheep which had been kept as reserves. In a second period between 13th December 1949 and 27th June 1950 the blood and bone marrow of twenty-eight ewes was examined. Two sheep were sampled on the first date mentioned, the next two on 11th January and thereafter in pairs at fortnightly intervals.

*Peripheral blood.* (January to July 1949)

The results obtained for the erythrocyte and leucocyte properties are shown in Tables 4 and 5 respectively. These results are expressed as mean values for the Groups B, C, D and R over all ages, (Col. *f*) and the means and S.E. (Mainland, 1938) for all groups and all ages, (Col. *g*). In the case of Groups B, C, D and R the mean values for P.C.V., Hb. and M.C.H.C. are shown for sheep of different ages; this subdivision on the basis of age is also shown for R.B.C., M.C.V., W.B.C. and D.L.C. in the case of Group B.

*Effect of age.* The mean values for the sheep of different ages in the four groups in respect of P.C.V. (Col. *a*) and Hb. (Col. *b*) show that the sheep under two years of age had lower values than sheep over that age. With the exception of Group R this was consistently true within all the Groups. The figures for the R.B.C. (Col. d) for

TABLE 4

ERYTHROCYTE PROPERTIES IN SHEEP OF DIFFERENT AGES IN GROUPS B, C, D, AND R*

| *Erythrocyte property* | *Group* | *No. of sheep* | *Age in years* | | | | | | | | *Mean for groups of all ages(f)* | *Mean and S.E. over all groups and all ages (g)* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Under 1* | 1 – 2 | 2 – 3 | 3 – 4 | 4 – 5 | 5 – 6 | 6 – 7 | 7 – 8 | | |
| P.C.V. % | B | 44 | 38.10 | 39.10 | 41.17 | 40.00 | 42.50 | 41.10 | 41.48 | — | 40.30 | |
| | C | 49 | 25.60 | 24.00 | 31.00 | 37.71 | 30.00 | 29.25 | 29.33 | — | 28.71 | 32.80 |
| | D | 64 | 29.70 | 30.35 | 31,20 | 31.31 | 33.19 | 34.08 | 30.80 | 32.25 | 31.27 | ±0.39 |
| | R | 37 | 31.23 | 32.00 | 32.25 | 32.25 | 31.00 | 34.50 | 30.00 | 33.50 | 31.70 | n=194 |
| | Mean for years over 4 groups (a) | | 31.07 | 31.23 | 34.10 | 33.55 | 34.33 | 35.12 | 33.91 | 32.5 | | |
| Hb. gms/100ml. | B | 47 | 11.27 | 11.60 | 11.76 | 11.51 | 12.12 | 11.94 | 12.10 | — | 11.72 | 9.94 |
| | C | 48 | 8.46 | 7.80 | 9.11 | 9.09 | 9.20 | 8.48 | 8.47 | — | 8.73 | ±0.10 |
| | D | 64 | 8.96 | 9.34 | 10.10 | 9.78 | 9.96 | 9.20 | 9.80 | 9.60 | 9.49 | n=192 |
| | R | 33 | 9.84 | 10.03 | 9.95 | 10.90 | 9.50 | 10.45 | 9.80 | 11.00 | 10.00 | |
| | Mean for years over 4 groups (b) | | 9.65 | 9.62 | 10.33 | 10.17 | 10.23 | 9.41 | 10.22 | 9.90 | | |
| M.C.H.C. % | B | 44 | 29.10 | 29.40 | 28.35 | 28.30 | 28.10 | 27.60 | 28.88 | — | 28.29 | 30.37 |
| | C | 48 | 33.60 | 32.60 | 29.07 | 29.71 | 30.83 | 29.00 | 30.78 | — | 29.98 | ±0.20 |
| | D | 64 | 30.18 | 30.80 | 32.70 | 31.56 | 30.38 | 27.17 | 31.90 | 29.94 | 30.53 | n=189 |
| | R | 33 | 31.79 | 31.58 | 30.75 | 33.00 | 30.50 | 30.25 | 32.50 | 33.00 | 31.50 | |
| | Mean for years over 4 groups (c) | | 31.18 | 31.03 | 29.95 | 30.29 | 30.00 | 28.12 | 30.48 | 30.60 | | |
| R.B.C. $10^6$/cu.mm. | B (d) | 44 | 10.72 | 10.54 | 11.58 | 10.82 | 11.55 | 10.93 | 10.74 | — | 10.97 | 10.97 ± 0.19 |
| | C x | 22 | | | | | | | | | 6.72 | |
| | D x | 24 | | | | | | | | | 8.03 | |
| | R x | 20 | | | | | | | | | 9.81 | |
| M.C.V. cu. μ | B (e) | 44 | 36.20 | 37.70 | 35.50 | 37.80 | 38.50 | 38.40 | 38.69 | — | 37.31 | 37.31 ±0.44 |
| | C x | 22 | | | | | | | | | 43.84 | |
| | D x | 24 | | | | | | | | | 40.00 | |
| | R x | 20 | | | | | | | | | 33.10 | |

x = Means within each group not calculated because number of observations too small.

n = Number of observations.

* Group B C D R

Month of Sampling January April June July

TABLE 5

LEUCOCYTES IN SHEEP IN GROUPS B, C, D, & R

| | *Mean values for group B* | | | | | | | *Mean values for groups (f)* | | | | *Mean and S.E. over all groups and all ages (g)* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Age in years* | | | | | | | | | | | | |
| | *Under 1* | 1 – 2 | 2 – 3 | 3 – 4 | 4 – 5 | 5 – 6 | 6 – 7 | B | C | D | R | *Mean* | *S.E.* ± |
| Leucocyte Count $10^3$/cu.mm. .. | 7.67 | 6.42 | 7.77 | 6.56 | 7.26 | 7.30 | 6.36 | 7.08 | 7.43 | 9.33 | 7.04 | 7.62 | 0.223 |
| Differential Leucocyte Count /cu.mm. | | | | | | | | | | | | | |
| Band Neutrophils .. .. | 35 | 50 | 29 | 46 | 17 | 31 | 41 | 37 | 13 | 11 | 5 | 20 | 3.3 |
| Lobulated Neutrophils .. .. | 2274 | 2317 | 2367 | 2437 | 1880 | 2574 | 2591 | 2412 | 3049 | 3767 | 2500 | 2845 | 122.7 |
| Eosinophils .. .. .. | 96 | 135 | 177 | 128 | 242 | 251 | 211 | 172 | 51 | 185 | 141 | 152 | 14.4 |
| Lymphocytes .. .. .. | 4881 | 3684 | 4781 | 2761 | 4894 | 4154 | 3238 | 4068 | 3791 | 5066 | 4062 | 4241 | 152.5 |
| Monocytes .. .. .. | 586 | 233 | 418 | 164 | 222 | 300 | 281 | 303 | 671 | 540 | 355 | 431 | 22.3 |

Group B also showed this effect. The mean values for M.C.H.C. for sheep in different ages (Col. *c*) showed that the sheep under two years of age had higher levels than sheep over this age, but within the Groups this was only true in the case of Groups B and C. The M.C.V. of the sheep under three years of age was lower than of sheep over that age. (Col. *e*). No effect due to age was apparent in either W.B.C. or D.L.C. in the sheep in Group B. (Table 5).

*Effect of season.* The mean values for P.C.V. and Hb. for the four Groups (*f*) showed Group B to be highest, Group C lowest and the levels for Groups D and R to occupy an intermediate position. This trend was evident for sheep of the same age in the four groups. The M.C.H.C. was lowest in Group B. There was no consistent trend within the other three groups. The highest W.B.C. was found in Group D and the D.L.C. showed the mean values for neutrophilic and eosinophilic leucocytes and lymphocytes to be higher in this group than in the other three groups.

The P.C.V., Hb. and M.C.H.C. measurements of the sheep sampled in January, April and June (Groups B, C and D) were submitted to an Analysis of Variance (Snedecor, 1946). The results from the sheep sampled in July (Group R) were omitted because of the small numbers of sheep in some age groups. In the case of P.C.V. and Hb. subdivision of degrees of freedom due to age showed that the significant effect was due to the low values for sheep under two years of age and for M.C.H.C. to the higher value recorded with sheep of this age.

In addition to this effect of age, differences due to month of sampling were also significant at 1 per cent level. Sheep bled in April and June had significantly lower P.C.V. and Hb levels than those sampled in January, while June levels were significantly higher than those found in April. The M.C.H.C. values in January were significantly lower than those of April and June.

*Effect of pregnancy.* Of the fifteen sheep between one and two years old in Group C (April) seven were pregnant and eight were non-pregnant. The mean figure for haemoglobin for the pregnant sheep was 7.9g./100 ml., which was lower than the figure of 8.5g./100 ml.—the mean for the non-pregnant sheep, but in a comparison of the means test, according to the method of Mainland (1938), 't' was found to be 0.391 and 'p' to be 0.7 showing the difference between the two means to be insignificant. Thus in the limited number of observations in which comparison can be made, pregnancy appears to exert no influence on Hb. level.

*Effect of worms.* The worm burdens of the sheep sampled in this investigation have been published by Morgan, *et. al.* (1951) and it is with their permission that the figures are used for correlation with the results of the haematological examination. The log of the

worm burden was plotted against the P.C.V. for each sheep in Groups B and C and Groups D and R and the result is shown in Figs. 2 and 3 respectively. The log of the worm burden was chosen

Fig. 2

in preference to the figure for the actual worm burden as this latter figure covered a very wide range. In Fig. 2 where worm burden (Log) is represented by x and P.C.V. by y, the regression line is given by y = 60.75 to 7.70 x. Thus when P.C.V. = 25.0, worm burden (Log) = 4.64; P.C.V = 36.0, worm burden (Log) = 3.21, and when P.C.V. = 40.0 worm burden (Log) = 2.69. The regression coefficient for worms (Log) on P.C.V. = —7.7032 and is significant at 1 per cent *viz* t = 8.37 and n = 91. Thus there was a negative association between the worm burden and the P.C.V. level in the case of the sheep in Groups B and C. No such association could be demonstrated in respect of Groups D and R. (Fig. 3).

Fig. 3

*Peripheral blood and bone marrow* (December 1949—June 1950)

The packed cell volume figure has been taken as representing the erythrocyte level in each sheep and Fig. 4 shows the fluctuations during the period of observation. The pattern of this variation is shown in Table 6 where the mean values for P.C.V., Hb. and R.B.C. are shown for three periods. The first period shows the highest levels, the second the lowest and for the third period the levels are intermediate. Fig. 4 shows that the fall in the P.C.V. coincided with the rise in worm burden up to 22nd March and that thereafter the worm burden and P.C.V. showed no relationship.

The mean corpuscular volume varied from 29.5 to 38.5 cu. mm. but this variation could not be related to the three periods of time shown in Table 6. The mean corpuscular haemoglobin concentration was remarkably constant ranging only from 27 to 31.5 per cent.

Fig. 4

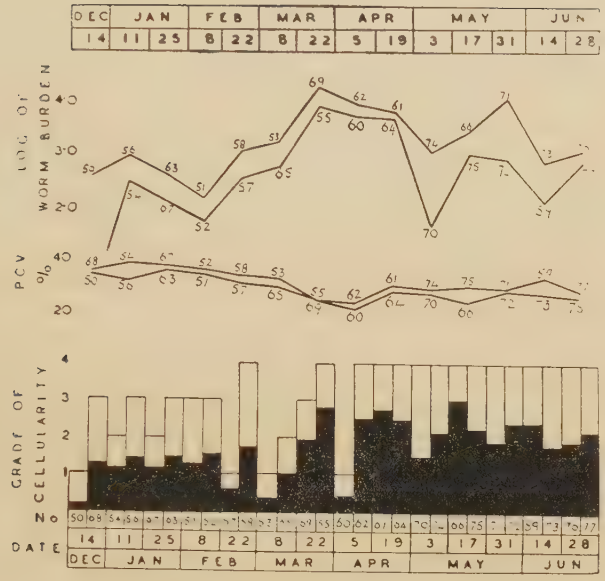


Fig. 4. Showing the variations in worm burden (log), packed cell volume and marrow picture of sheep sampled from 14th December 1949 to 28th June 1950.

□ Myeloid Tissue
■ Erythroid Tissue

The numbers on the lines of the figures correspond to the ear numbers of the sheep.

Table 6

| | *Number of sheep* | *P.C.V.%* | *Mean value and range for Hb. g./100ml.* | *R.B.C. $10^6$/cu.mm.* |
|---|---|---|---|---|
| Dec. 14th — Feb. 8th | 8 | 36.43 (33.0–39.0) | 10.55 (10.1–11.6) | 10.91 (10.2–12.1) |
| Feb. 22nd—April 5th | 8 | 29.0 (23.0–35.0) | 8.68 ( 6.9–10.8) | 8.31 ( 7.0–10.3) |
| April 19th—June 28th | 12 | 30.25 (28.0–35.5) | 8.98 ( 7.3–10.8) | 8.80 ( 7.6–10.7) |

The differential leucocyte counts showed a tendency for the neutrophilic leucocytes to be more numerous from March 22nd to April 19th. No evidence of regenerative changes in the form of punctate basophilia and polychromasia could be found in any of the blood films examined.

In the lower half of Fig. 4 the M/E ratio has been superimposed on the cellularity grading so as to express an absolute measurement of the erythroid tissue present as opposed to the relative measurement given by the M/E ratio alone. It will be seen that with the exception of sheep No. 60 the animals sampled on and after March 22nd showed more erythroblastic tissue than those sampled before that date.

The maturation curves for all the sheep, except No. 60 showed a fairly constant distribution of the erythroblasts of different ages and there was no shift to the left to coincide with the rise in erythroblastic tissue referred to above, nor was this rise accompanied by an increase in mitotic activity. The m.c.s for the neutrophilic granuloblasts showed more variation from sheep to sheep, but the variation could not be correlated either with the time of sampling or with the numbers of neutrophilic leucocytes in the peripheral blood. In sheep No. 60 there was a very low incidence of both primitive erythroblasts and primitive granuloblasts and a preponderance of adult forms in both series.

## DISCUSSION

There is no difference in the erythrocyte levels of sheep at one and at two years old and this agrees with the findings of Holman (1944), but the fact that sheep under two years of age gave lower levels than older sheep has not been previously reported. The sheep under two years of age carried a higher worm burden and such sheep are acknowledged to be more susceptible to the effect of helminth infestation. These facts may account for their lower erythrocyte levels.

Erythrocyte levels are shown to have fallen in April (Table 4) and between February 22nd and April 5th (Table 6). Holman (1944) in a study of the seasonal variation of the erythrocyte levels of sheep on low ground pasture, also reports a significant fall in February and March but by April the levels had again started to rise. This earlier recovery in levels may be related to the earlier improvement to be expected on low ground as compared with hill pasture.

Holman (1950) appears reluctant to regard the seasonal oligocythaemia which may occur in herbivorous animals as pathological. When, however, the mean values shown for P.C.V., Hb. and R.B.C. in Col. *f* of Table 4 are compared with the normal erythrocyte values for sheep obtained by other workers only the figures for Group B (January) show any close agreement; the figures for the remaining groups (April, June and July) are all lower. It has been shown that the values obtained in June and July were significantly lower than those in January and that the values in April were significantly lower than those in June and July. Thus if the January values represent the normal range then at least in the case of the sheep sampled in April there is an anaemia. Examination of the marrow shows that until 22nd March there is a tendency towards an erythroblastic hypoplasia of which the marrow picture in sheep No. 60 is an advanced stage.

The increase in erythroblastic tissue in the marrow which can be demonstrated in sheep sampled after 22nd March (Fig. 4), as compared with sheep examined before that date, is not accompanied by a shift to the left in the maturation curves of the erythroblasts as might be expected to occur in a marrow responding to blood loss.

The anaemia noted in this investigation occurs at a time when a state of malnutrition exists in Scottish hill sheep (Orr and Fraser, 1932; Morgan, *et. al.*, 1951). However, the worm free sheep on a low plane of nutrition (Group B, Table 2) which suffered an average weight loss of 14 per cent showed no reduction in erythrocyte levels. Meteorological data from an observatory a few miles from the farm showed that during March, as compared with the previous two months, there was a rise in the mean monthly temperature and in the hours of sunshine and a reduction in the number of days on which ground and air frost were recorded. From these facts it is reasonable to assume that there was an improvement in the pasture during the month. There was more erythroblastic tissue in the marrow of the sheep sampled on and after 22nd March than in the sheep sampled before that date (Fig. 4). It is suggested that this change is related to the improvement in the pasture and that conversely the tendency towards erythroblastic hypoplasia in the period before 22nd March is related to the low plane of nutrition persisting at that time.

Hawkins, Cole, Kline and Drudge (1944) and Hawkins and de Frietas (1947) have shown that fluctuations in the erythrocyte level may be related to variations in worm egg count in sheep on pasture and the results of this investigation confirm their findings. Morgan, *et. al.* (1951) showed that the worm burden in these sheep consisted mainly of members of the *Trichostrongylidae* with *Ostertagia spp.* and *Trichostrongylus axei* predominating : both these species are known to include anaemia among their pathogenic effects (Morgan and Hawkins, 1949). To show they were present in sufficient numbers to be of pathogenic importance in this investigation use was made of an index for the assessment of pathogenicity of worm burdens designed by Morgan and Gordon (1951). In Group B only one of the forty-four sheep carried a worm burden of 'probably pathogenic significance'; in the forty sheep in Group C this figure was twenty-two; and of the one hundred and one sheep in Groups D and R only thirteen had significant worm burdens. In the case of the sheep sampled at fortnightly intervals (Fig. 4) eight of sixteen sheep sampled on or after the 22nd March carried worm burdens of pathogenic significance.

From this evidence it is suggested that the lower erythrocyte levels demonstrated in the sheep Group C as compared with those in Group B (Table 4), and in the sheep sampled from 22nd Feb-

ruary to 5th April (Table 6) as compared with those sampled before 22nd February, are related to the higher worm burden present at these times. It has been argued that a state of malnutrition existed in the sheep during this period and it is known that under these conditions parasites exert their greatest effect on erythrocyte levels (Lawrence, Groenwald, Quin, Clark, Ortlepp and Bosman, 1951).

It is suggested that the absence of close association between worm burden and P.C.V. in the sheep sampled in late April, May, June and July was due to the improved nutritional status of the sheep from mid-April onwards. The exact method by which anaemia is produced by *Trichostrongylus* and *Ostertagia spp.* has not been determined, but in neither case can the reduction in erythrocyte levels be entirely explained on the basis of blood loss.

The findings described here confirm the view that parasites exert their maximum effect on sheep on a low plane of nutrition. This effect is probably achieved by interference with the synthesis or absorption of a factor necessary for normal erythropoiesis. This factor was related to nutrition as when nutrition was at a high level the effect of the worms was partly offset. Even in sheep on a low plane of nutrition it was still present in adequate amounts in the absence of worms, but the effect of even small helminth infestations on its availability (as seen by the reduction of erythrocyte levels) indicated its presence in minimal amounts when nutrition was low. The exact nature of this factor cannot be suggested. It may be included among the 'specific nutritive factors', the existence of which was postulated by Stoll (1940) in a discussion of the 'break through in immunity' caused by poor nutrition.

## CONCLUSIONS

A diet providing half maintenance was shown to have no effect on haematopoiesis in worm free sheep.

A significant association has been demonstrated between the erythrocytic oligocythaemia and the rise in worm burden in a heft of Scottish hill sheep in late winter and early spring.

The marrow of the sheep in this heft was shown to have less erythropoietic tissue from January to mid-March than from mid-March to June.

## ACKNOWLEDGMENT

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## REFERENCES

Coffin, D. L. (1945). *Manual of Veterinary Clinical Pathology.* Comstock Publishing Co. Inc; Ithaca, N.Y.

Gordon, H. McL., and Whitlock, H. V. (1939). *J. Counc. sci. industr. Res. Aust.*, **12**, 50.

Grunsell, C. S. (1951). *Brit. vet. J.*, **107**, 16. (1955). *J. comp. Path.*, **65**, 8.

Hawkins, P. A., Cole, C. L., Kline, E. E., and Drudge, J. H. (1944). *Vet. Med.*, **39**, 154.

Hawkins, P. A. and de Frietas, M. C. (1947). *J. Parasit.*, **33**, Suppl. 22.

Holman, H. H. (1944). *J. comp. Path.*, **54**, 26, 40; (1950). Chapter in *Diagnostic Methods in Veterinary Medicine* by G. F. Boddie. Oliver & Boyd; Edinburgh.

Lawrence, G. B., Groenwald, J. W., Quin, J. I., Clark, R., Ortlepp, R. J., and Bosman, S. W. (1951). *Onderst. J. vet. Sci.*, **25**, 1.

Mainland, D. (1938). *The Treatment of Laboratory & Clinical Data.* Oliver & Boyd; London.

Morgan, B. B., and Hawkins, P. A. (1949). *Veterinary Helminthology* Burgess Publ. Co.; Minneapolis.

Morgan, D. O., and Gordon, H. McL. (1951). Personal communication.

Morgan, D. O., Parnell, I. W., and Rayski, C. (1950). *J. Helminth.*, **24**, 101; (1951). *Ibid.*, **25**, 177.

Morgan, D. O., and Sloan, J. E. N. (1947). *Scot. Agric.*, **27**, 28.

Orr, J. B., and Fraser, A. H. H. (1932). *Trans. Highl. agric. Soc. Scot.*, **44**, 64.

Snedecor, G. W. (1946). *Statistical Methods.* Iowa State College Press; Iowa.

Stoll, N. R. (1940). *J. Amer. vet. med. Ass.*, **96**, 305.

Whitby, L. E. H., and Britton, C. J. C. (1942). *Disorders of the Blood.* Churchill; London.

Wintrobe, M. M. (1946). *Clinical Haematology.* Kimpton; London.

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# MALIGNANT NEOPLASIA OF THE OESOPHAGUS AND RUMEN OF CATTLE IN KENYA

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## INTRODUCTION

This communication records the finding of a high incidence of neoplasia of the oesophagus and rumen in a few herds of indigenous, Zebu-type African cattle maintained by natives of the Masai tribe in a small, remote locality of Kenya Colony. The cause remains undetermined and this note is published because further work by the present author will not be possible and also as being of interest to medical or veterinary oncologists elsewhere.

## FIELD OBSERVATIONS

The locality in question is a small valley running down an escarpment at an altitude of 8,000 to 9,000 feet. Above the escarpment is dense rain forest; below it the country is intersected by numerous valleys with a predominantly moorland type of vegetation, all apparently similar to the affected one. The Masai have probably utilised this region for grazing their herds since the end of World War I; they are a semi-nomadic, pastoral tribe with considerable familiarity with a wide range of animal diseases, e.g. anthrax, black-quarter, trypanosomiasis, East Coast fever, rinderpest and pleuro-pneumonia. This is a fact of some importance, as for much of the past history and for estimates of the recent incidence they are the only possible source of information.

The Masai and veterinary "scouts" of this district are insistent that the condition is peculiar to the one valley; this avowal accords well with the information available to the local veterinary officer in the field, to whose notice the disease was first brought. It appears to have been recognised as a distinct entity since about the year 1935 and it must be allowed, as will be shown later, that the clinical and post mortem features are not such as could be easily confused with those of any other disease. Since 1935, the incidence of the condition has increased, particularly after 1942. Healthy cattle if removed from the affected locality are said never to acquire the disease, but if sick animals are transferred elsewhere it follows its invariably fatal course. No new foci of "infection" have arisen as the result of the introduction of diseased stock and it is claimed that the affected area can be sharply delineated from adjacent "clean" grazing.

It is of interest to note that, whilst the herds of cattle involved are self-contained, they are periodically trekked to an earthlick thirty to

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forty miles distant. The geophagia which is widespread amongst cattle in this area is probably associated with phosphorus deficiency, but the latter is not so severe as to cause skeletal lesions.

*Total Incidence*

The figures which follow are those obtained by careful questioning of the herd owners; many of the affected animals were probably destroyed for meat when it became obvious that they had developed the incurable condition. It was affirmed that lesions of characteristic appearance could always be found on slaughter.

Herd I consisted of approximately one hundred animals. On 13th August, 1952, losses were said to have numbered thirty-five in the preceding year. Two animals showing characteristic symptoms were examined; of these, one (Cow 1) was slaughtered and material taken for examination.

Herd II comprised approximately seventy cattle; deaths in the preceding year had risen to twenty. One animal which had died was also autopsied on 13th August, and material sent to the laboratory (Cow 2).

Herd III consisted of about 20 animals. One animal out of twenty died in the year prior to 13th August. A second one with characteristic clinical signs was examined on 19th September and the diagnosis confirmed post mortem (Cow 3). Herd IV consisted of about fifty animals. Four were lost in the year preceding 13th August, and one sick animal with the usual clinical appearance was seen on 19th September.

If it is agreed that only animals seen by a veterinary surgeon can be accepted as clinical cases of neoplasia then the total actually observed on two occasions at five weeks interval (13th August to 19th September) was five out of an approximate total of 250 cattle, or two per cent. Of these it must be admitted that only three were confirmed histologically. Using the data provided by the owners, annual losses attributed to this condition varied between ten and twenty-five per cent approximately; there is little doubt that the higher figures are somewhat inflated, not because of inability to differentiate the neoplastic condition from others, but merely due to limitations of native arithmetic. It appears to be a reasonable deduction that annual losses might amount to as much as ten per cent or more of the total herds.

*Age and incidence*

The disease is confined to adult cattle of both sexes. The Masai say they have observed the disease in animals only three years old but personal observations indicate that the majority of cases are in cows which have had at least three or four calves—that is at least seven to eight years old.

*Symptoms and Course*

There is a progressive loss of condition with the skin tight and the hair-coat becoming harsh and dry. The visible mucosae may be abnormally pale and some cases show a voracious appetite. The most characteristic feature is a chronic rumenal tympany, easily visible as a distension of the left flank, which is doubtless due to disturbance of the mechanism of regurgitation either by infiltration of the oesophageal wall, by projections into its lumen or by involvement of the oesophageal groove.

Death always supervenes, if the animal is not slaughtered, usually after a period of illness lasting at least six months and sometimes up to two or three years.

## DESCRIPTION OF CASES

*Cow* 1. This was one of two sick animals from herd I produced for examination on 13th August. It was about ten years old and had had three calves; clinical signs of the disease had been present for seven months. At postmortem examination small papillomata were found down the whole length of the oesophageal mucosa. The rumen contained much earth and there was a heavy infestation of paramphistome flukes—a very common finding in Kenya. The rumenal contents were watery. Two large, blackened, ulcerated lesions were noted in the anterior part of the wall of the rumen, adjacent to the oesophageal groove. There were no significant lesions of any other organ—apart from cachectic changes. Microscopically, the granulomatous masses which formed the base and edge of the ulcerated areas consisted of well delineated cords and rounded masses of polygonal and squamous neoplastic cells embedded in a dense, fibrous matrix. In some cases the centre of these rounded foci consisted of a keratinised or caseous mass which fell out easily during manipulation of the section. A portion of a lymph node attached to the affected part of the rumenal wall also showed fibrosis and many similar neoplastic deposits (Fig. 1).

Sections of areas of the rumenal wall passing through the edge of neoplastic lesions showed the continuity of the surface epithelium with that which had already become malignant; the adjacent areas showed infiltration by cords of neoplastic cells penetrating to the outer layers of smooth muscle. There were also widespread precancerous changes in the stratified squamous epithelium—thickening and the production of large numbers of darkly staining, elongated papillae composed of narrow, columnar or spindle-shaped elements thrusting down into the submucosa and raised excrescences with early malignant change (Fig. 2).

Sections of prescapular lymph node, spleen and kidney presented no significant abnormality. Some blood vessels in the liver, especially the portal veins, contained abnormally large numbers of neutrophil polymorphs.

*Cow* 2. This was an animal from herd II which died during the night of 12th August. It was estimated to be about fifteen years old and had had nine calves; the duration of the disease was said to be three years.

There were "papillomatous" growths down the whole length of the oesophagus, some of them very large; a few were also found in the pharynx. An extensive ulcerated lesion involved the rumenal wall and the terminal portion of the oesophagus. A portion of the inferior third of the latter was fixed and sent to the laboratory. The thickness of the wall was greatly increased (up to about 2 cm.), showing infiltration of the muscle by a firm whitish tissue. The internal surface showed numerous irregular excrescences covered by an abnormally thick white mucosa. Some of these projections were pedunculated with a fibrous centre and 1 cm. or more long. There was one horn-like growth, extensively keratinised and measuring $2\frac{1}{2}$ x 1 cm., situated approximately 10 cm. from the cardiac opening (Fig. 3).

The rumenal contents were again watery. No metastatic lesions were noted in lymph nodes but many old calcified foci were seen in the liver. These were not submitted for examination and may have been parasitic. Haemorrhages were noted on the serosal surface of the large intestine; they were possibly evidence of a terminal peritonitis.

Microscopically the thickening of the oesophageal wall was found to be due to infiltration and destruction of the muscle bundles by strands of dense granulation tissue including islets of carcinomatous epithelium. The fibrous strands often contained numerous small blood vessels and heavy lymphocyte and polymorph infiltrations, whilst the larger carcinomatous nodules commonly showed extensive central keratinisation. There were numerous situations in which it was quite clear that the carcinomatous down-growths were continuous with greatly thickened surface epithelium.

*Cow* 3. This aged animal was destroyed on 19th September.

The oesophageal mucosa had a slimy, whitish deposit, easily scraped off, and the normal glistening surface was absent. In the upper third there were a few small papilloma-like lesions. The oesophagus was somewhat dilated and its wall flabby.

The rumenal contents were watery and not macerated as normally. A large thickened ulcerated area was found in the region of the anterior groove, 13 x 9 cm. in its greatest diameters and approaching to within 2 to 3 cm. of the cardiac opening (Fig. 4). This area was blackened and covered with a slimy, foul-smelling deposit. At the centre, the thickness of the rumenal wall was about 8 cm. of which the superficial half was whitish, friable, deeply fissured and obviously necrotic. The outer part was more fibrous with embedded nodules of a firm whitish appearance. This area was outwardly adherent to the diaphragm and one or two small nodules of lymphoid appearance were found in the fibrous tissue forming the adhesion. The mucosa adjacent to the ulcerated area had lost its typical papillae and was either granular in appearance or, especially towards the cardia, provided with irregular, firm whitish excrescences up to 1 cm. in diameter. The abomasal contents closely resembled those of the rumen, thus indicating a severe disturbance of rumination and digestion. A careful search failed to reveal metastatic lesions in the associated lymph nodes.

Histological examination of the floor of the ulcerated area showed deep necrosis, haemorrhage and infection. Beneath this was a layer consisting of solid cords of polygonal epithelial cells with relatively little supporting connective tissue (Fig. 5). The neoplastic cell cords did not show

keratinisation but their central elements were sometimes undergoing hydropic change and infiltration by neutrophil polymorphs. At the edge of the ulcerated area they could be seen to under-run the mucosa and to infiltrate through and between the muscle layers. The surface epithelium adjacent to the ulcer was often thickened or showed the production of large numbers of darkly staining, branching and delicate projections growing down into the lamina propia. There were numerous foci of intra-epithelial "pearl" formation (Fig. 6).

Microscopically the oesophageal mucosa in non-papillomatous areas showed thickening, hyperkeratinisation and a ragged, detaching surface layer. These changes presumably accounted for the loss of the normal glistening appearance. Sections of liver, kidney, prescapular and rumenal lymph nodes and adrenal gland revealed no significant abnormality. The spleen showed heavy haemosiderin deposits.

## DISCUSSION

In addition to the personal observations recorded here, the Masai owners affirm that papilloma-like lesions are present with great regularity in the oesophagus, and occasionally also in the pharynx, of animals they recognise as suffering from this disease complex. They regard the large blackened, ulcerated masses in the anterior part of the wall of the rumen as invariable. If it should be doubted that the observations of a primitive African people are reliable enough to carry weight, there is to support them the remarkable fact that they produced for examination four live sick animals and one dead one, all of which they said were suffering from a disease entity which was peculiar to this district. Of these five, three came to autopsy by a veterinarian and all were confirmed histologically as cases of malignant neoplasia. It is unfortunate that difficulty of access and other factors beyond our control have precluded further visits to the affected locality, in order to confirm the reported figures.

The gross and microscopic changes found in the oesophagus and anterior wall of the rumen indicate that large areas of the stratified squamous epithelium lining these structures, possibly also the pharynx, show "precancerous" changes such as thickening, hyperkeratinisation, papilloma formation, etc., and that the malignant growths are definitely multicentric in origin.

No plants or parasites were found which could be regarded with suspicion and the limited distribution of the disease would perhaps contra-indicate such an explanation. Little is known of the geology of the region, but Pulfry (1953) states that it is a tertiary volcanic formation in which it is most unlikely that abnormally high concentrations of radioactive minerals would be detected.

There are indications in the relevant literature that carcinoma of the rumen, usually stated to involve the anterior part of its walls in the vicinity of the oesophageal groove, is by no means a rare con-

dition in aged cattle (Feldman, 1932). German authorities (Joest, 1937; Nieberle and Cohrs, 1949) are sufficiently familiar with this neoplasm to state that it may assume two forms—a flattened, ulcerated, scirrhous type, corresponding to those described here and a broad-based, projecting type superficially somewhat papillomatous in appearance. Kitt (1950) gives some figures suggesting that carcinoma of the rumen constitutes about 5 per cent of all such neoplasms in the bovine. It is also interesting to note that in the horse a similar figure is given by this author for carcinoma arising in the pars oesophagea of the gastric mucosa; Kitt (1921) and Nieberle and Cohrs (1949) make the observation that when carcinoma of the oesophagus occurs in the horse it is usually in the thoracic part and may be associated with epithelioma of the pars oesophagea of the stomach. The similarity to Cow 2 is obvious, but none of the authors quoted appears to know of a comparable case in the bovine. Although Gastrophilus larvae have been claimed by some to be of aetiological significance in the horse the frequent occurrence of the parasite would lead one to expect a higher incidence of gastric carcinoma if this were true. The same applies to paramphistome flukes in cattle.

Papillomata of the oesophagus, as is well-known, are not uncommon in cattle. They may be single or multiple, localised or diffuse, and extend into the pharynx and mouth cavity (Kitt, 1921; Nieberle and Cohrs, 1949). Both of these authorities suggest an infective, presumably viral, origin. In the cattle described here it is apparent that the incidence was very high and that the papillomatosis probably represented an early phase in the stimulation of the epithelium to malignant neoplastic development.

## CONCLUSIONS

In a small locality of the Masai reserve of Kenya five cases of a distinctive chronic disease were brought for examination within a time interval of five weeks. Three of these were examined postmortem.

All three were confirmed as cases of rumenal, or oesophageal and rumenal, carcinoma of multicentric origin.

Figures reported by the herd owners indicate that annual mortality due to this condition may reach ten per cent.

Clinical and pathological features of the disease are described.

Preliminary observations have failed to indicate the carcinogenetic factor or factors involved.

## ACKNOWLEDGMENTS

I am deeply grateful to Mr. B. Bagenal, M.R.C.V.S., who first brought this condition to my notice and provided details of the postmortem examination of Cows 1 and 2 and some of the field history.

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## REFERENCES

Feldman, W. H. (1932). *Neoplasms of Domesticated Animals,* W. B. Saunders Co.; Philadelphia.

Joest, J. (1937). *Handbuch der Speziellen Pathologischen Anatomie der Haustiere.* 2nd Edition, Richard Schoetz; Berlin.

Kitt, T. (1921). *Pathologische Anatomie der Haustiere.* 5th Edition, Ferdinand Enke; Stuttgart.

Kitt, T. (1950). *Lehrbuch der Allgemeinen Pathologie für Tierärtze.* 7th Edition. Ferdinand Enke; Stuttgart.

Nieberle and Cohrs (1949). *Lehrbuch der Speziellen Pathologischen Anatomie der Haustiere,* 3rd Edition. Gustav Fischer; Jena.

Pulfrey, W. (1953). Personal communication.

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## LEGENDS TO FIGURES

FIG. 1. Cow 1. Carcinoma deposits in a rumenal lymph node. H. and E. (× 400).

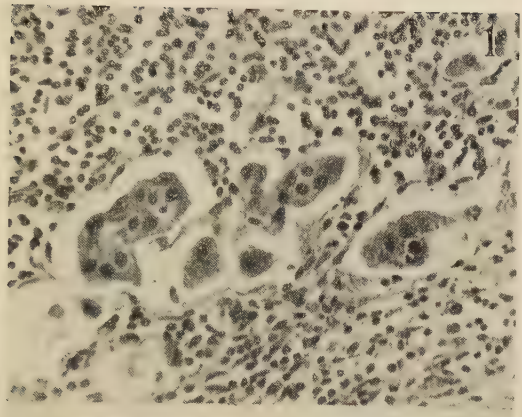
FIG. 2. The edge of a small rumenal "excrescence" in Cow 1. Normal epithelium on the extreme right. Masson's trichrome stain (× 45).

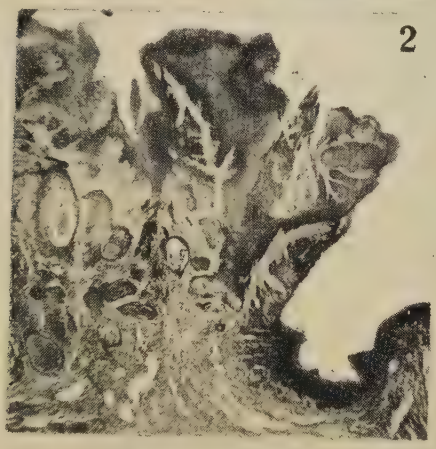
FIG. 3. Terminal part of oesophagus of Cow 2. The larger divisions of the scale represent cms. There is gross thickening of the wall by carcinomatous tissue; the thickened white mucosa can be easily seen towards either end on the left; see also the horn-like growth superiorly, and numerous mucosal excrescences.

FIG. 4. Rumenal lesions of Cow 3. The scale on the left represents centimetres. The oesophageal opening is on the left in the middle. The thickness of the wall and deep superficial necrosis is obvious in the deep transverse incision.

FIG. 5. Cow 3. Neoplastic cell cords in the depths of the ulcerated area of the rumenal wall; some central cells show degeneration but there is no keratinisation. Zenker-formol, Haematoxylin-eosin-azure (× 200).

FIG. 6. Rumen of Cow 3. Production of branching, tenuous and darkly-staining epithelial downgrowths. Several intra-epithelial "pearls." H. & E. (× 150).





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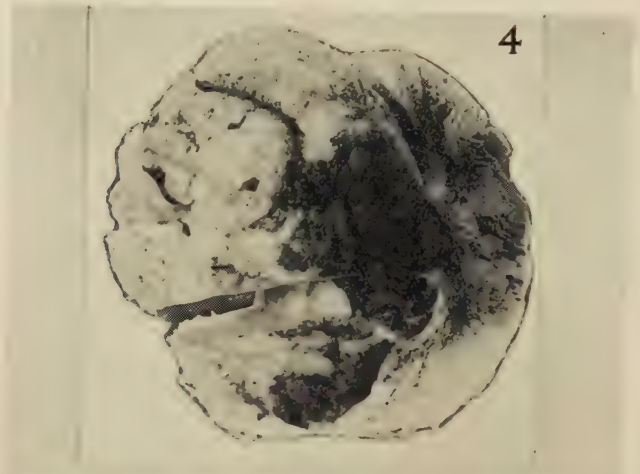
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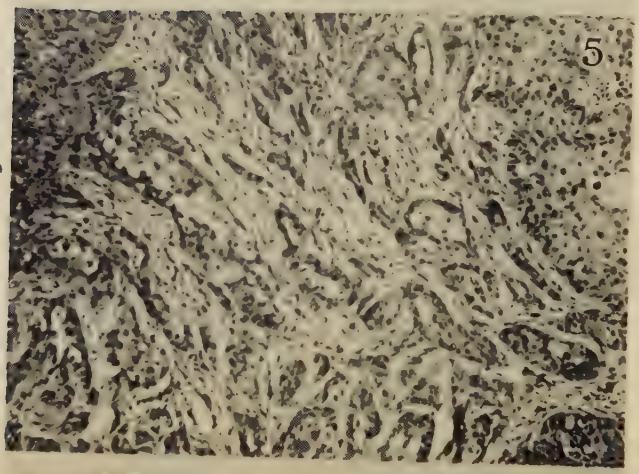
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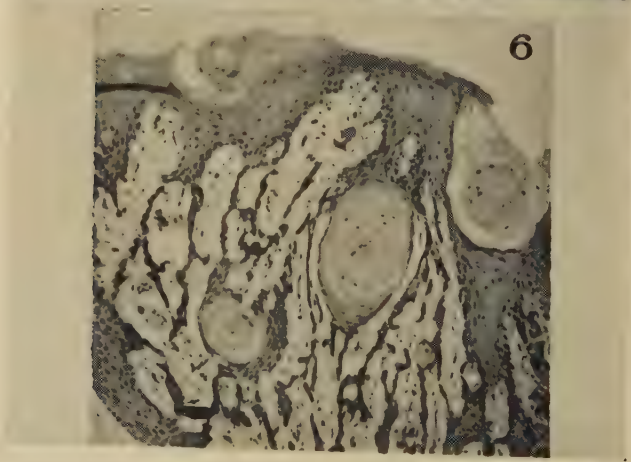
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# MELANOTIC TUMOURS OF DOGS.

By

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## INTRODUCTION

During the period 1950-53 a total of 1,150 neoplasms from dogs have been examined in the Department of Pathology, Royal Veterinary College (Cotchin, 1954). Of these, 101 (8.8 per cent.) were melanotic tumours, that is to say, they contained variable amounts of brown pigment, the melanotic nature of which in doubtful cases was confirmed by showing it to be bleachable and iron-free. They were distributed as follows :

| | | |
|---|---|---|
| Eye | | 3 |
| Mouth | | 15 |
| Skin | | 83 |
| Malignant | 20 | |
| Baso-squamous | 2 | |
| Benign cellular | 28 | |
| Benign fibrous | 33 | |
| | | 101 |

## SEX AND AGE INCIDENCE

*Sex*

Of 96 animals whose sex was recorded, 70 (73 per cent.) were males and 26 females. In the whole series, the sex of 1,001 animals was known : 508 (51 per cent.) were male, or, not counting animals bearing tumours of the genital system, 431 of 658 (65.5 per cent.) were male. There is thus little evidence of any very special sex incidence of melanotic tumours in the dog.

*Breed*

Of 100 animals whose breed was recorded 30 were Scottish terriers, 27 were mongrels or crossbreds (21 and 6), 11 Spaniels and 6 Dachshunds. The remaining 26 were spread over a number of breeds, none being affected more than four times (Airedale, Retriever 4; unspecified terrier 3; Sealyham, Cairn, Bull terrier 2; Great Dane, Dalmatian, Greyhound, Kerry Blue, Irish Setter, Corgi, Dandie Dinmont, Pug, Collie 1). In the whole series of dogs the breed of 1,037 was known : 28 per cent. were mongrels, 15 per cent. Spaniels and 10 per cent. Scottish terriers. There is thus an indication that the Scottish terrier is rather unduly prone to bear melanotic tumours.

*Age*

The age recorded for 92 animals with melanotic tumours ranged from 3 to 17 years, with an average of 8.8. This was practically the same as the average age (8 years) of 1,018 dogs in the whole series for which the age was known.

## DISTRIBUTION AND NATURE OF TUMOURS

### *Eye*

There were 3 specimens (cases 1 to 3). One was a melanoma of the corneo-scleral junction, extending half way over the cornea from the upper edge. The second and third were black, friable tumours occupying the globe of the eye in a 13-year-old mongrel dog and an aged terrier dog respectively. These two melanomas were both in eyes that were removed operatively and the aged terrier at any rate showed no sign of metastasis or recurrence one year later. On the other hand, a more recent specimen of melanoma in the globe of the eye showed a marked histological appearance of malignancy and metastases were later found at post mortem examination.

In the two melanomas of the globe, the tumour cells have large, round or oval vesicular nuclei, often with a prominent single nucleolus (Fig. 1). The outline of most of the cells is indistinct and they do not stain readily with haematoxylin and eosin. Some of the cells show a polygonal shape and some have short cytoplasmic processes. The cells are arranged in rows, or rather irregularly, with a tendency to a whorled grouping; beneath the cornea the cells tend to lie at right angles to the direction of the rows of cells between the stromal fibres, which are generally scanty. The cells usually contain only moderate amounts of melanin but large rounded heavily-pigmented cells are present, forming in some places groups in which the cell details are obscured. A few mitoses are present.

### *Mouth*

Details of the 15 cases (4 to 18) are shown in Table 1.

TABLE 1

MELANOTIC TUMOURS OF THE MOUTH OF DOGS

| *Case No.* | *Age (years)* | *Breed* | *Sex* | *Site* | *Post-mortem examination made* | *Site of metastases* |
|---|---|---|---|---|---|---|
| 4 | 11½ | Scottie | f. | Lip | | (Regional nodes clinically enlarged) |
| 5 | 8 | Dachshund | f. | Gum | + | Lungs. Local tumour recurred 7 months after removal |
| 6 | 12 | Scottie | f. | Gum | + | |
| 7 | 12½ | Sealyham | m. | Tonsil | + | Regional nodes |
| 8 | 9 | Scottie | m. | Hard palate | + | |
| 9 | 11 | Wire-haired fox terrier | f. | Soft palate | + | Regional nodes |
| 10 | 9 | Great Dane | m. | Lip | + | Regional node |
| 11 | 8 | Spaniel | m. | Cheek | + | Regional node |
| 12 | | Scottie | | Gum | | |
| 13 | Aged | Scottie | m. | Gum | | |
| 14 | 11 | Mongrel | m. | Gum | | |
| 15 | 15 | Mongrel | m. | Cheek | | |
| 16 | 13 | Mongrel | m. | Hard palate | + | |
| 17 | 13 | Spaniel | m. | Cheek | + | Lungs |
| 18 | 10½ | Mongrel | f. | Cheek | + | Regional node |

Of the 15 melanotic tumours in this group, 5 arose on the gum, 4 on the cheek, 2 on the lip, 2 on the hard palate, one on the soft palate and one in the tonsillar region. The age of the dogs, which was known for 13 animals, ranged from 8 to 15 years, average 11. There were 5 Scottish terriers, 4 Mongrels, 2 Spaniels and single Dachshund, Sealyham, Terrier and Great Dane. Nine of the animals were known to be male and five female. Post-mortem examination was made of ten of the dogs. Metastases were found in 7: in five cases they were present in the mandibular or superior cervical lymph nodes and in 2 cases in the lungs. Some of the lung metastases were pigmented, while others were not.

These oral melanomas were ulcerated lesions, which invaded the submucosal and muscular tissues (Fig. 2). The colour was generally not uniformly black throughout the tumour: one tumour (case 5) appeared macroscopically white in colour although the metastases in the lungs were partly black.

The histological structure is variable but in general the tumour cells tend to be either spindle-shaped (Fig. 3) or to have a more polygonal shape (Fig. 4) and to be more epithelial in appearance. The spindle-shaped cells when small tend to have darkly-staining nuclei, while when large their nuclei are more vesicular and often show a prominent single nucleolus. Some of the spindle-shaped cells are long and others appear to form part of long ribbon-like rows of cells. The tumour cells are sometimes loosely arranged, irregularly or in bundles, or they form more or less closely-packed interwoven bundles. The cells may be loosely grouped, forming sometimes ill-defined whorls. One of the tumours (case 8) appears very like a neurofibrosarcoma. The polygonal cells usually have rather large vesicular nuclei, often with a single prominent nucleolus; they tend to be arranged to form ill-defined acini among the scanty stromal fibres (Fig. 5) and the cells sometimes lie rather transversely in their rows (Fig. 6). In some of the tumours, both types of cell—spindle-shaped or polygonal—are present and transitional types are seen. The tumours are all histologically malignant, as judged by the presence of mitoses, invasive growths and resemblance to known malignant tumours. They also resemble the malignant tumours of the skin, with perhaps a more marked tendency to have a spindle-cell structure.

Metastasis was usually by way of the lymph stream. One of the tumours (case 18) reduced considerably in size under X-ray therapy but the local lesion was not completely freed from tumour cells and in any event the mandibular node was also involved.

### *Skin*

*Epithelial Tumours*

Two examples were examined.

Case 19. A subcutaneous tumour from the head of a 5-year-old Collie dog was oval in shape, with a well-defined edge, and measured

$2\frac{1}{2}$ x $1\frac{1}{2}$ x 1 cm. It was black in colour, with a central bulging discharging point on the skin.

This is a melanotic baso-squamous tumour, with cornification. The tumour (Fig. 7) is rather lobulated, with cords of cells, one or more cell thick, forming a closely-packed complicated folding pattern and marked squamous differentiation is present, usually in the centre of the epithelial masses. Melanin is present in the epithelial cells, some of the pigmented cells being dendritic in shape, in the cornified material and in the stroma (Fig. 8).

Case 20. The second tumour, from a 5-year-old Spaniel dog, was oval in shape, measuring 3.5 x 1.5 cm.; it lay subcutaneously, being covered by stretched hair-bearing skin. In section, it is a pigmented sweat-gland tumour with mucoid and myxomatous change.

Neither of these tumours appeared to be malignant. The pigmentation seems to be merely incidental to the development of an epithelial tumour.

The remaining cutaneous melanotic tumours, 81 in number, could be grouped as being definitely or probably malignant, or benign or probably benign.

*Malignant and probably malignant melanotic cutaneous tumours*

Details of the 20 cases (21 to 40) are given in Table 2. The known malignant ones, shown first, were those in which there was post-mortem evidence of metastasis. The probably malignant ones, shown next, were histologically similar to the known malignant ones and showed clinical evidence of being malignant locally, although they were not known definitely to have metastasized, except in one case (40) where a lymph node biopsy was examined.

TABLE 2

MALIGANT AND PROBABLY MALIGNANT CUTANEOUS MELANOTIC TUMOURS

| *Case No.* | *Age (years)* | *Breed* | *Sex* | *Site* | *Site of metastasis* |
|---|---|---|---|---|---|
| (i) Known malignant tumours | | | | | |
| 21 | 12 | Airedale | m. | Over prepuce | Regional nodes |
| 22 | 4 | Mongrel | m. | Scrotum | Widespread |
| 23 | | Dalmatian | f. | Side of neck | Lungs |
| 24 | 12 | Sealyham | m. | Digit | Regional nodes, lungs |
| 25 | 10 | Mongrel | m. | Scrotum | Regional node |
| 26 | 9 | Dachshund | f. | Over mamma | Regional nodes, lungs |
| 27 | 6 | Airedale | m. | Over prepuce | Widespread |
| 28 | 9 | Mongrel | m. | Digit | Widespread |
| (ii) Local clinical malignancy, and histological malignancy | | | | | |
| 29 | 7 | Scottie | m. | Digit | |
| 30 | 10 | Mongrel | m. | Scrotum | |
| 31 | 12 | Spaniel | m. | Digit | |
| 32 | 4 | Greyhound | m. | Metatarsus | |
| 33 | 8 | Scottie | f. | Over mamma | |
| 34 | | Cross-bred | m. | Scrotum | |
| 35 | 8 | Bull terrier | f. | Digit | |
| 36 | 10 | Scottie | m. | Circumanal region | |
| 37 | 9 | Scottie | m. | Scrotum | |
| 38 | 17 | Dachshund | f. | Over mamma | |
| 39 | 8 | Mongrel | m. | | |
| 40 | 8 | Scottie | m. | (Lymph node biopsy) | |

The age of 18 of the 20 dogs was known, ranging from 4 to 17 years, average 9. Fifteen of the animals were male and five female. Five of the animals were Scottish terriers: it may be noted that these bore tumours diagnosed histologically as malignant. Five were mongrels and one was a cross-bred, other breeds affected being Dachshund (2), Airedale (2), Spaniel (1), Greyhound (1), Bull terrier (1), Sealyham (1) and Dalmatian (1). The site of the tumour was in 5 cases the scrotum and in 5 cases a digit. Other sites affected were : over the mamma (3), prepuce (2), side of neck (1), metatarsal region (1) and circumanal region (1). Eight of the dogs were examined post-mortem and were found to show metastases. In 7 cases the regional nodes were affected and in 7 cases the lungs. In 3 dogs, the metastases were present in other parts of the body. In case 22 they were found in the lungs, subcutaneous fat of right thigh, flank, axilla, kidneys, brain, thyroid glands, myocardium, intestinal wall, mediastinum and bone marrow of humerus and femur. In case 27, both superficial and deep inguinal nodes, lungs (Fig. 9), kidneys (Fig. 10), mesentery, pancreas, liver, peri-prostatic fat, right temporal and right flank muscles, subcutaneous tissue of back and above left stifle, parietal pleura, mediastinum, suprasternal node and bone marrow of humerus, femur and vertebral bodies (Fig. 11) were affected. In case 28 the right popliteal node, lungs, myocardium, mediastinum and anterior mediastinal node were involved. In the dog, as in man, the metastases from a malignant melanoma appear to grow well in more or less any tissue.

These malignant melanomas appeared to develop deep in the cutis and to grow both deeply into the underlying tissue and out towards the surface, usually with ulceration. However, even when definitely malignant, the tumour may have had a well-defined edge macroscopically. The degree of melanin pigmentation was rather variable and was usually not equal throughout any given tumour but it was, as a rule, quite heavy. Metastases were also usually heavily pigmented, but sometimes, as in the lungs, pigmented and unpigmented tumours or parts of tumours might lie side by side.

Histologically, the malignant or probably malignant melanotic tumours of the skin of the dog have a varied structure, but a number of features are commonly observed. The nuclei are typically oval, rather palely-staining and have a single—or, if the nucleus is large, a double—nucleolus. The cells vary in shape but tend to form groups, or rows, and there is a tendency to a swirling (Fig. 12), knotted or whorled pattern to appear in these rows. In the rows, the cells sometimes lie across the direction of the rows and sometimes appear as though clasping a neighbouring tumour cell. The cells are either spindle-shaped and then are either short or long, or they form long ribbon-like rows of cells (Fig. 13), or they are

more angular (Fig. 14). sometimes with short or medium-length processes (Fig. 15). There is a tendency for these more epithelial-appearing cells to be loosely arranged in alveoli; sometimes they show a certain tensity of patterned arrangement recalling a mosaic and sometimes they resemble in arrangement structures which could be considered to mimic "nerve-ending" figures (Fig. 16), structures which are also seen in some of the benign tumours. The degree of pigmentation of the individual cells varies from slight to heavy and there is often a grouping of the more heavily-pigmented cells around the less heavily-pigmented ones. These more heavily-pigmented cells, in these as well as in the benign tumours, often show a nuclear conformation in bleached sections (Fig. 17) which suggests that they are also melanin-forming cells and not merely melanin-laden phagocytes. Mitoses are often numerous. Although the surface epithelium is often lost from ulceration, it is present in some instances; no definite origin of the tumour cells from the epidermis can be seen, although in occasional places the appearances are quite suggestive of this.

*Benign and probably benign cutaneous melanotic tumours*

These tumours were classified as benign or probably benign chiefly because of their clinical appearance. In general, they were smaller than the malignant tumours. They did not show invasive growth but rather tended to grow outwards to form a bulging dome-shaped tumour on the skin surface, or to be more frankly pedunculated. In some cases, too, there was a long post-operative history of non-recurrence. Histologically, the tumours tend to show the usual features of benign lesions, e.g. lack of signs of rapid growth or of tissue invasion, but some of them tend to a malignant appearance and the decision as to whether or not they should be classified as benign is then largely influenced by the macroscopical features.

These benign melanotic tumours fell broadly into one of two main groups—those that were predominantly cellular, and those that were predominantly fibrous. In some cases, multiple tumours were present on the same dog and they might be of one or other type, and some tumours were partly fibrous and partly cellular. The possibility exists that the fibrous tumours were in some cases actually neurofibromas.

*Benign cellular melanomas.* Details of the 28 cases (41 to 68) are shown in Table 3.

TABLE 3

BENIGN MELANOMAS OF THE SKIN OF THE DOG

| *Case No.* | *Age (years)* | *Breed* | *Sex* | *Site* |
|---|---|---|---|---|
| 41 | 13 | Irish Setter | f. | Digit |
| 42 | 9 | Spaniel | f. | |
| 43 | 5 | Spaniel | f. | |
| 44 | 9 | Scottie | f. | Perineum |
| 45 | 4 | Scottie | f. | Digit |
| 46 | 4 | Mongrel | m. | Axilla |
| 47 | | Scottie | | Digit |
| 48 | | Corgi | | Digit |
| 49 | 5 | Scottie | m. | Digit |
| 50 | 8 | Mongrel | m. | Digit |
| 51 | 10 | Mongrel | m. | Digit |
| 52 | 14 | Scottie | f. | |
| 53 | 6 | Labrador | f. | |
| 54 | 7 | Mongrel | m. | Digit |
| 55 | 11 | Retriever | m. | |
| 56 | 6 | Dandie Dinmont | f. | Digit |
| 57 | 8 | Spaniel | m. | Digit |
| 58 | 10 | Retriever | m. | Digit |
| 59 | 9 | Scottie | f. | |
| 60 | 8 | Spaniel | m. | Digit |
| 61 | 4 | Airedale | m. | Digit |
| 62 | 8 | Dachshund | m. | Elbow |
| 63 | 3 | Pug | m. | |
| 64 | 8 | Retriever-cross | m. | Digit |
| 65 | 6 | Airedale | m. | Metatarsus |
| 66 | 6 | Mongrel | m. | Eyelid |
| 67 | | Dachshund | m. | Digit |
| 68 | 10 | Scottie | m. | Temple |

Of 26 of the 28 dogs, 17 were males and 9 were females. The age of 25 of the dogs ranged from 3 to 14 years, average 8. Seven of the dogs were Scottish terriers, 5 mongrels, and 4 Spaniels. The site of 21 of the tumours was specifically stated by the clinician and of these 15 were on the digits, thus providing a striking contrast, as will be seen later, with the site of origin of the fibrous melanomas.

These benign cellular melanomas appeared to develop in the deeper layers of the dermis and to grow outwards, bulging the skin upwards, sometimes with ulceration, to form a hemispherical or dome-shaped structure. On occasion there was obvious pedunculation but the tumours tended to lie flatly on the skin and sometimes a fair amount of tissue was submerged below the general skin surface, usually having a well-defined edge macroscopically against the surrounding tissue. The tumours were generally heavily pigmented. The tumours appeared to grow outwards between hair-follicles but one or more of the hair-follicles might be included in the growth (Fig. 23). The skin was generally thin and stretched but sometimes there was marked epidermal hyperplasia, occasionally with a suggestion of continuity with the tumour cells. There was sometimes a zone of dermal fibrous tissue between the tumour and the surface epithelium.

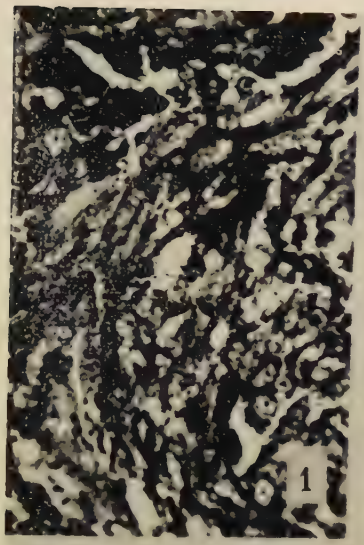
The amount of fibrous tissue present is variable but is usually very scanty. The melanin pigmentation is usually heavy; some of the cells are very densely pigmented and blocky in shape but they have a typical nuclear structure, with a prominent nucleolus. The cells resemble narrow or plump fibroblasts, or are polygonal with short processes, or are rather plump, or elongated and ribbon-like, or obviously dendritic. The melanin granules may lie in rows in the large ribbon-like cells and the latter may be multinucleated. There is a tendency for some cells to be heavily pigmented, while neighbouring cells are more or less free of melanin. The cells may lie closely packed (Fig. 18), or lie in rows, often orientated towards the surface of the tumour and these may appear "caught up", or form loose swirls or whorls (Figs. 19, 20). Sometimes structures to some extent recalling nerve-ending figures are seen (Fig. 21) and sometimes groups of cells are formed resembling the whorls of an epithelioid meningioma. Mitoses are generally scarce. Some tumours are very vascular. There is no definite capsule but the edge of the tumour may be well-defined.

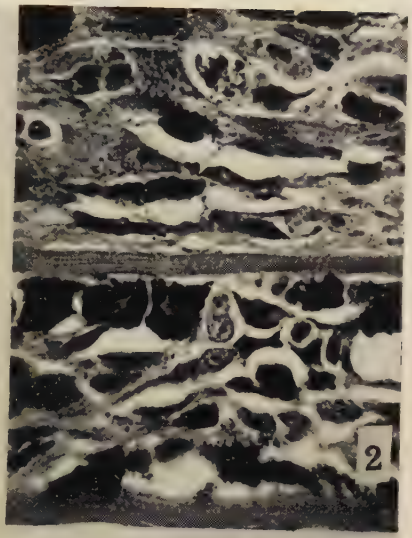
As previously stated, in classifying these tumours as benign, account was taken of their macroscopic features and absence of definite positive information as to recurrence or metastasis, but some of the tumours had a histology suggesting malignancy. Others tended towards the fibrous type in structure.

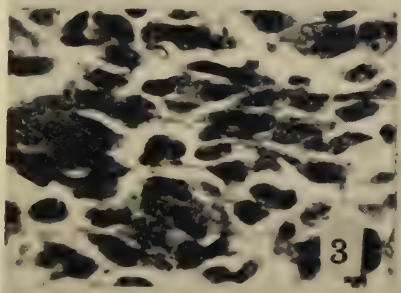
*Benign fibrous melanomas.* Details of the 33 cases (69 to 101) are shown in Table 4.

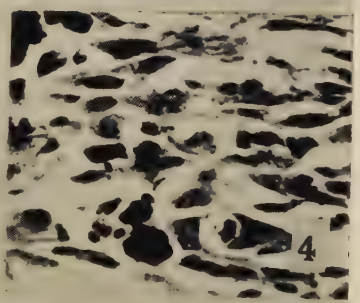
Of the 33 animals, 25 were known to be male and 7 female. The age of the dogs ranged from 3 to 15 years, average 9. Thirteen of the affected animals were Scottish terriers and 10 were mongrels or crossbreds. No other breed was represented more than 3 times. Four of the tumours were on the scrotum and 4 on the prepuce. Eleven of the tumours were on the limbs but only one was definitely known to be on a digit: the infrequency with which lesions of this kind were reported on the digit is in striking contrast to the benign cellular melanomas. The structure of these benign fibrous tumours was variable. The lesions were dome-shaped or more freely pedunculated (Fig. 22) and their cut surfaces varied in colour from white through grey to black and were not often uniformly pigmented.

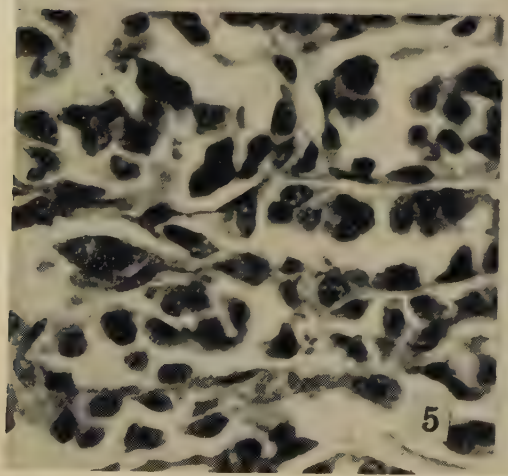
The general picture is of a whorled fibrous tissue, in which melanin-forming cells appear to be an integral part, growing up from the deepest level of the dermis and displacing, or sometimes including, hair follicles (Figs. 24, 25). The surface epithelium is sometimes stretched thin, or it shows marked hyperplasia, especially at the edge of an ulcer. There is sometimes a zone of non-neoplastic fibrous tissue between the tumour and surface epithelium. The amount of pigment in the surface epithelium varies from little to



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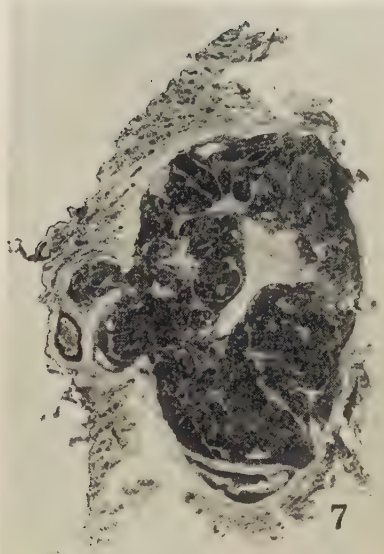

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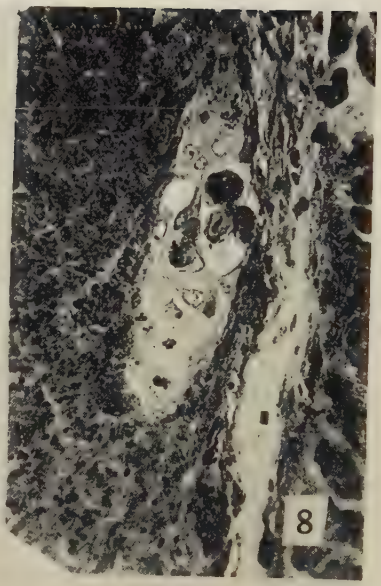

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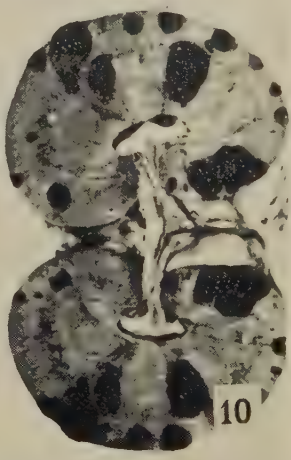

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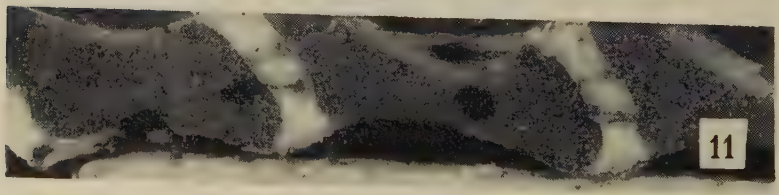

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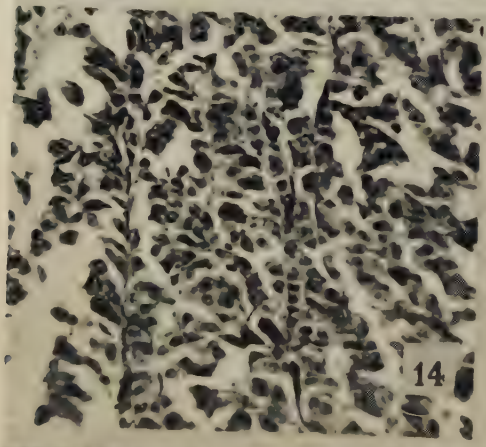
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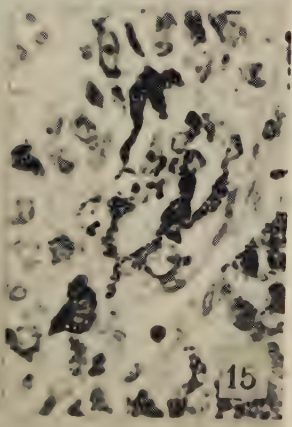
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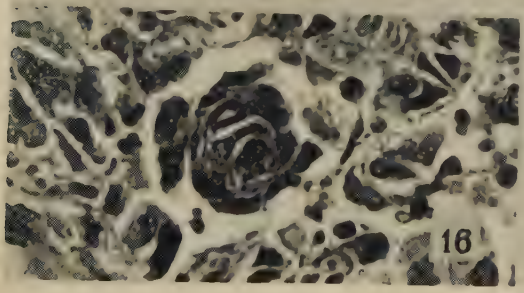
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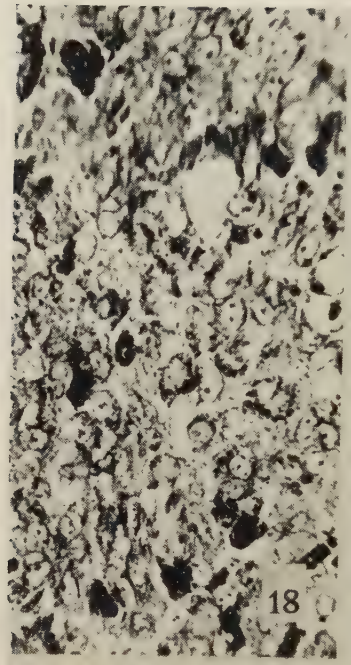
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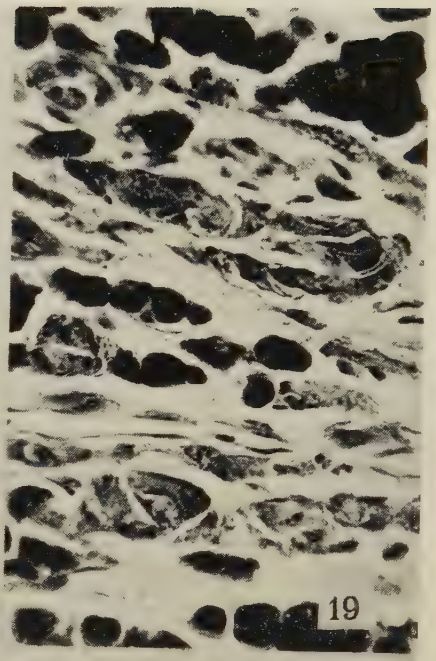
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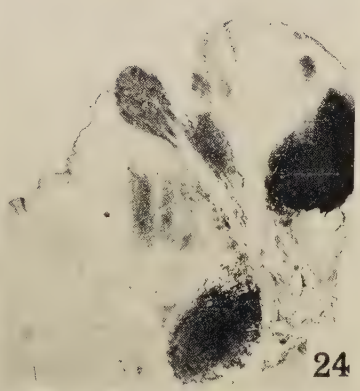

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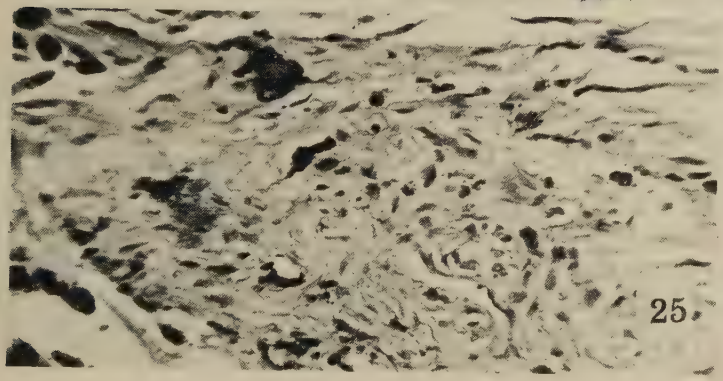

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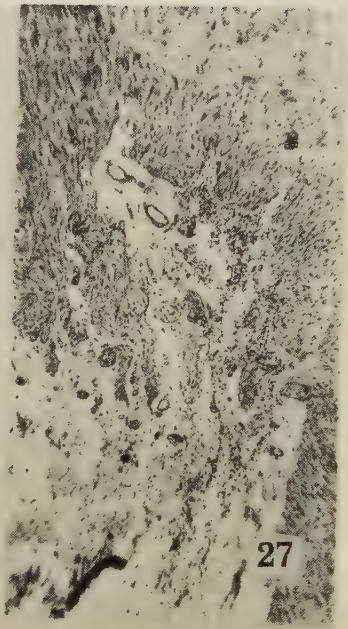
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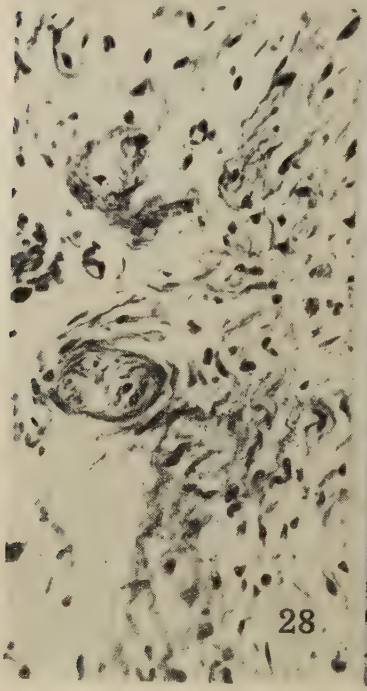
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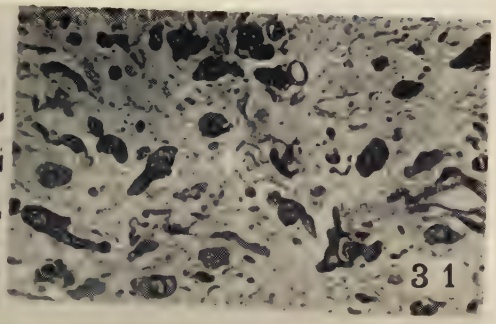
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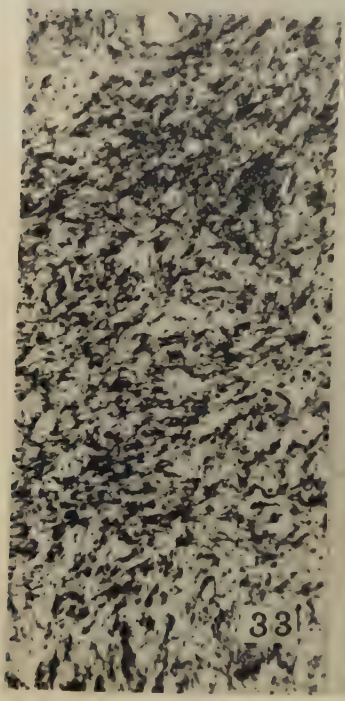
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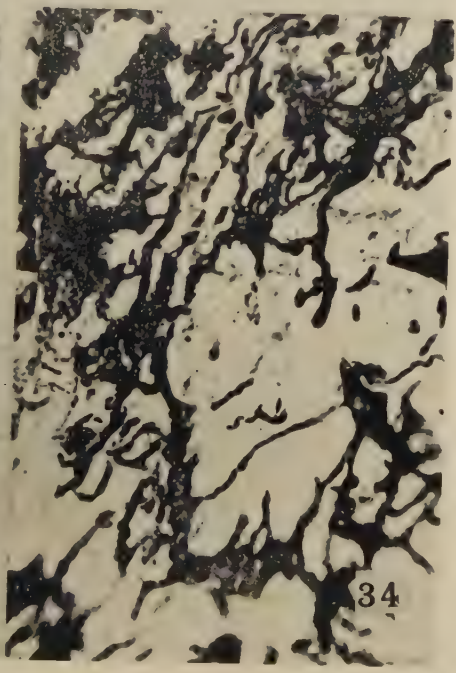
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TABLE 4

BENIGN FIBROUS MELANOMAS OF THE SKIN OF THE DOG

| *Case No.* | *Age (years)* | *Breed* | *Sex* | *Site* |
|---|---|---|---|---|
| 69 | 3 | Scottie | f. | Digit |
| 70 | 6 | Alsatian-cross | m. | Left carpus |
| 71 | 8 | Mongrel | m. | |
| 72 | 10 | Kerry Blue | f. | Mammary region |
| 73 | 9 | Mongrel | m. | Carpus |
| 74 | 9 | Mongrel | f. | Stifle |
| 75 | 7 | Scottie | m. | Elbow |
| 76 | 7 | Scottie | m. | Foreleg |
| 77 | 7 | Scottie | m. | Prepuce |
| 78 | 10 | Mongrel | m. | Prepuce |
| 79 | 11 | Scottie | m. | Leg |
| 80 | 6 | Mongrel | m. | |
| 81 | 6 | Dachshund | f. | Hock |
| 82 | 11 | Scottie | m. | Widespread |
| 83 | 9 | Scottie | m. | Leg |
| 84 | 11 | Retriever | m. | Foreleg |
| 85 | 5 | Spaniel | m. | Scrotum |
| 86 | 14 | Cairn | m. | Right and left flank |
| 87 | 10 | Cross-bred | m. | |
| 88 | 8 | Bull terrier | m. | |
| 89 | 6 | Scottie | m. | Prepuce |
| 90 | 14 | Terrier-cross | m. | Over triceps |
| 91 | 11 | Beagle-cross | m. | Face |
| 92 | 7 | Spaniel | f. | Mammary region |
| 93 | 15 | Cairn | m. | Throat region |
| 94 | 12 | Mongrel | f. | Leg |
| 95 | 7 | Scottie | m. | Metacarpal region |
| 96 | 12 | Fox terrier | f. | Temple |
| 97 | 7 | Scottie | m. | Scrotum |
| 98 | 4 | Scottie | | Hock |
| 99 | 9 | Scottie | m. | Scrotum |
| 100 | 10 | Scottie | m. | Prepuce |
| 101 | 10 | Spaniel | m. | Scrotum |

much, and obvious isolated or grouped melanoblasts can be seen, particularly in the basal layers of the epithelium. The fibrous tissue, which stains sometimes well, sometimes poorly, with van Gieson, tends to show a knotted or whorled arrangement (Fig. 26) and in some instances this fibrous tissue appears continuous with the sheaths of small nerves at the base of the tumour (Figs. 27, 28). Some of the pigmented cells, from the surface epithelium or the hair follicles, appear to sink into the underlying connective tissue, or even into the tumour itself, which may contain few other pigmented cells (Fig. 29).

The pigmented cells in the tumour resemble narrow or plump fibroblasts (Fig. 30) or are long ribbon-like cells with blunt processes (Fig. 31). There is a tendency in some tumours for the formation of structures recalling abnormal "nerve-ending" figures, the cells of which are sometimes heavily pigmented (Fig. 32). The nucleus of the fibroblast-like cells, if small, is moderately darkly staining but the larger cells of this type, and the other pigmented cells, have

pale oval nuclei with a single prominent nucleolus. Some unpigmented cells have similar nuclei. There are in some areas whorled groups of cells, recalling the epithelioid type of meningioma. In some tumours, there are densely or loosely felted networks of dendritic melanotic cells (Figs. 33, 34).

## DISCUSSION

Most of the reports of melanotic tumours in dogs refer to skin lesions, although Sastry (1953), who reviews the literature, concluded that the eye and the soft tissues around the oral cavity are particularly prone to be affected by melanomas. Tumours in the eye have been reported, for example, by Michael (1948), McClelland (1941) and Bloom (1942), and in the mouth by Povar and Povar (1950). The most extensive histological account is that by Mulligan (1949), who classifies the melanotic tumours of the dog as "cancerous" or "non-cancerous", that is, as malignant or benign. He states that the "non-cancerous" melanomas may be transformed into "cancerous" melanomas when irritated or incompletely removed but it is difficult to see how proof of this could be obtained. He saw 35 cases, occurring mostly in what he terms the "canine cancer age", from 7 to 14 years. Breeds with moderately pigmented skin, especially the Airedale, Scottish terrier, Springer Spaniel and Boston terrier, were particularly affected. Common sites for the tumour, in his experience, were the head, thorax and extremities, with a particular proneness of the eyelids and the soft tissues around the buccal cavity, such as the cheeks and lips. He says that two or more neoplasms were present in over 10 per cent. of cases. Metastases were usually found in the lungs, less commonly in the spleen, heart, liver, intestines, pancreas, peritoneum and pleura. Mulligan describes the "non-cancerous" (benign) tumours as showing cells which are polyhedral, stubby spindle, long spindle or slender-tapered in shape, and also dendritic, and heavily laden with melanin granules. The polyhedral cells are arranged in solid masses, in alveolar configuration or in lobulated groups. The spindle-tapered cells show a long bundled pattern and may be intimately associated with small cutaneous nerves and recall the structure of proliferated nerve sheath cells, with nuclear palisading. Whorls of cells mimic the structure of specialized nerve endings. The nuclei are practically always solitary, varying in shape from round or oval to oblong. The filamentous chromatin is regularly distributed around a single discrete small nucleolus. In the malignant ("cancerous") melanomas, nearly all the cells are polyhedral or stubby; dendritic cells are rare. The cells are quite loose and set in patterns. Structures resembling nerve sheath tumours are lacking. The nuclei may be swollen, and two or more prominent nucleoli may be seen; mitoses may be bizarre.

Ottosen (1947) described melanotic tumours of the skin and cutaneous membranes in dogs. He saw 15 cases, 14 occurring on the skin and one in the mouth. The head and the limbs were the regions most affected and in several instances the tumours were multiple. In 2 of 9 cases in which a post-operative history was obtained, there was evidence of malignancy. Ottosen's cases seem to correspond with the benign cellular and malignant forms described in this paper.

The varied histological structure of the melanomas of dogs has thus long been recognized. It seems that the "cancerous" and "non-cancerous" types described by Mulligan correspond to the malignant and the benign cellular types here described, while perhaps the benign fibrous melanomas would only in part fall in his "non-cancerous" group. Spurrell (1932) has described lesions apparently corresponding to the benign fibrous type. He, however, considered them to be neurofibromas which were only secondarily pigmented by a sinking-in of pigment cells from the overlying epithelium, and stated categorically that there was no evidence in his series that the tumour was connected with pigment formation and the title of melanoma was not justified. However, the sinking-in of pigment cells does not seem to account for all the pigment cells in the benign fibrous melanomas. The skin tumours, recorded by Gray (1900), are of this type and were classified by M'Fadyean as pigmented fibromas.

These benign fibrous melanomas, from their whorled structure and from the presence of groups of cells which might be considered to resemble nerve-ending figures, or the whorls of epithelioid meningiomas, might provisionally be considered to be pigmented neurofibromas but before this can be finally accepted the constant demonstration of nerves and nerve sheaths in relation to which they are arising will be necessary. In these tumours, some of the melanotic cells present are clearly adventitious, seeming to invade the fibrous tumour from the overlying epithelium or to spread from included hair follicles, but in most cases this does not seem to be an adequate explanation for the number and distribution of the pigmented cells, which give the impression of sometimes being an integral part of the tumour. The fibrous tissue component of the lesions, even if it outweighs the pigmented tissue, may actually be a later development as a stage in the evolution of the tumour, or on the other hand it may be merely a reaction of the dermis to the presence of melanin-forming or melanin-bearing cells. Study of much earlier lesions than those examined in this series will be needed to elucidate this point and particularly to throw light on the exact origin of the melanoblasts in the various types of melanotic tumour

It is suggested that the various histological pictures of these melanomas can best be understood by assuming that they all reflect the diverse activities of a single type of cell, the melanoblast. As

regards man, it is generally agreed that the human cutaneous melanoblast is normally restricted to the basal-cell layer of the epithelial structures of the skin and that the ordinary pigmented mole and the malignant cutaneous melanoma (Cunningham, 1952; Willis, 1953) arise from a migration of these cells from the epithelium into underlying dermal connective tissue; Masson (1951) particularly has considered the role of proliferating Schwann cells of the dermal nerves in forming part of the naevus. Thus the normal human cutaneous melanoblast is apparently of ectodermal type and the mole and the malignant melanoma can be classified as ectodermal tumours. Where do these melanoblasts come from? In lower species—amphibia, chick, and mouse (Becker, 1948)—the melanoblast has been shown to be a derivative of the neural crest and to reach its final position by a process of migration, which would suggest that at some stage this ectodermal melanoblast is in a mesodermal position. This neural crest origin has not yet been demonstrated for the dog but it seems very possible that both in dog and in man this origin obtains.

True mesodermal melanoblasts of unspecified origin are said to be present normally in the skin of the ape (Becker, 1948), and Webb and Calhoun (1954), in describing the histology of the skin of the dog, say that in many sections pigment-laden cells are seen in portions of dermis near to the basement membrane. It will be necessary to determine whether these particular cells, which might perhaps be of importance in giving rise to melanotic tumours, are true mesodermal melanoblasts, or epidermal melanoblasts, that have migrated into the dermis or even merely melanin-laden phagocytes. In man, mesodermal melanoblasts are thought to occur in two structures only—the so-called Mongolian spot and the blue naevus of Jadassohn-Tieche. The former structure (Allen, in Anderson, 1949. p. 1243) is found in the sacral region in the last half of embryonic life or the first year of infancy, disappearing thereafter. The blue naevus (Montgomery and Kahler, 1939) appears as a flat or slightly elevated blue or bluish-black lesion, occurring particularly on the trunk and extremities. These two lesions—Mongolian spot and blue naevus—are structurally the same, being composed of interlacing fasciculi of spindle-cells with long cytoplasmic processes and oval fibrocytoid nuclei. If the pigment were not present, Allen states, the histological picture would be that of a dermal neurofibroma, and he thinks that the basic morphogenesis of the pigmented lesion and of neurofibromas are similar. The blue naevus differs from the ordinary pigmented mole in that the pigmented cells lie more deeply in the dermis, that these cells do not show a definite epithelial origin and that the blue naevus rarely gives rise to malignant tumours. The benign melanotic fibrous tumour of the dog's skin might have its human counterpart in the blue naevus.

Whether or not the ectodermal and the mesodermal melanoblasts of man are different cells, or merely represent the same cells in different locations, is not clear. Harvey, Dawson and Innes (1940), in referring to human melanoblasts, say that they are almost always epidermal in location, and raise a question as to whether this is an advance in status, or due to a disappearance of a dermal melanoblast of lower vertebrates. It is possible to consider epidermal and mesodermal melanoblasts as being actually the same cell, originally arising from the neural crest, but to suppose that in man, migration of these cells to their definitive position in the epidermis is the rule, the mesodermal position persisting only in the Mongolian spot and the blue naevus, where the migrating neural-crest derivatives are, as it were, left behind.

If the melanomas of the dog arise from a single type of melanoblast the problem of their histogenesis can be studied, firstly, in very early lesions, secondly, by determining whether or not mesodermal melanoblasts exist which may give rise to some or all of the melanotic tumours, and thirdly, by experimentally attempting to produce melanotic tumours. Passey (1938) applied an extract of gasworks tar once a week to the skin of the base of the neck of 9 dogs, persisting with the application for a period of 7 years. Three tumours appeared on the skin, none of them before the end of the sixth year of tarring. One of the tumours was a malignant melanoma, the second probably was a malignant melanoma, while the third was reported to be jet black in colour. Passey sounded a note of caution in interpreting these findings, stating that it remained to be determined whether the induction of melanomas in this experiment was merely fortuitous. However, it does seem possible that the application of a potent carcinogen, such as methylcholanthrene, to the skin of a dog of a breed apparently highly susceptible to melanoma formation, such as the Scottish terrier, at a site prone to melanoma formation, such as the digit or scrotum, may be helpful in elucidating the histogenesis of canine melanotic tumours.

## CONCLUSIONS

An account is given of 101 spontaneous melanotic tumours of dogs. Three of these affected the eye, and 15 arose in the mouth. Of the remaining 83 cutaneous tumours, two were of epithelial type, 20 were malignant, and 61 were benign or probably benign. These latter 61 were further classified as being of benign cellular (28) or benign fibrous type (33). There was no sharp line of demarcation between the three main types of skin melanomas described.

Study of early lesions, supplemented by attempts at experimental reproduction, will be necessary to elucidate the histogenesis of these melanotic tumours.

## REFERENCES

Anderson, W. A. D. (1949). *Pathology*. Henry Kimpton; London.
Becker, S. W. (1948). in : *Biology of Melanomas*. p. 82 Ed : Miner, R. W. and Gordon, M. Academy of Sciences; New York.
Bloom, F. (1942). *J. Amer. vet. med. Ass.* **100**, 439.
Cotchin, E. (1954). *Brit. vet. J.*, **110**, 218, 274.
Cunningham, J. A. (1952). in : *Progress in Fundamental Medicine*. p. 28 Ed : J. F. A. McManus. Henry Kimpton; London.
Gray, H. (1900). *J. comp. Path.*, **13**, 254.
Harvey, W. F., Dawson, E. K., and Innes, J. R. M. (1940). *Debatable Tumours*. p. 27 Oliver and Boyd; Edinburgh.
Masson, P. (1951) *Cancer* **4**, 9.
McClelland, R. B. (1941). *J. Amer. vet. med. Ass.*, **98**, 504.
Michael, S. J. (1948). *Ibid.*, **113**, 253.
Montgomery, H., and Kahler, J. (1939). *Amer. J. Cancer*, **36**, 527.
Mulligan, R. M. (1949). *Amer. J. Path.*, **25**, 339.
Ottosen, H. E. (1947). *Skand. VetTidskr.*, **37**, 207.
Passey, R. D. (1938) *J. Path. Bact.* **47**, 349.
Povar, M. L., and Povar, R. (1950). *J. Amer. vet. med. Ass.*, **117**, 223.
Sastry, G. A. (1953). *Indian vet. J.*, **29**, 482.
Spurrell, W. R. (1932). *J. comp. Path.*, **45**, 172.
Webb, A. J., and Calhoun, M. L. (1954). *Amer. J. vet. Res.*, **15**. 274.
Willis, R. A. (1953). *Pathology of Tumours*. p. 899 Henry Kimpton; London.

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### Legends to Illustrations

1. Case 2. Melanoma of the globe of the eye. Note closely packed ill-defined cells, with variable melanin pigmentation. The nuclei are oval, pale, and contain a single prominent nucleolus. c.f. fig. 18. ×360
2. Case 13. Malignant melanoma of the gum spreading to the buccal mucosa, showing invasion of cheek muscle. Note the variable pigmentation of the cells, their angular shape and the presence of mitoses. × 650
3. Case 17. Malignant melanoma of the cheek, showing spindle-shaped cells. × 650
4. Case 13. Malignant melanoma of the gum, showing angular cells and some heavily melanotic cells. × 360
5. Case 9. Malignant melanoma of soft palate, showing ill-defined alveolar arrangement of cells. × 650
6. Case 11. Malignant melanoma of cheek, showing tendency for tumour cells to lie transversely in rows. × 650
7. Case 19. Baso-squamous melanotic epithelial tumour. Low power view, showing position of tumour. The oval structure just below the surface at the left mid-point is the discharging pore. × 4
8. Higher magnification of the same tumour as Fig. 7 showing squamous differentiation among basal cells, and melanin in epithelial cells and in stroma. × 260
9, 10, 11. Case 27. Metastases of malignant melanoma of prepuce in lungs, kidneys, and vertebral bodies.
12. Case 30. Malignant melanoma of scrotum, showing a swirling group of cells, most of which contain little or no melanin. × 260
13. Case 25. Malignant melanoma of scrotum, showing long ribbon-like, partly multinucleated, cells. × 650

14. Case 24. Malignant melanoma of digit. Epithelial-like cells with loose alveolar arrangement. × 260

15. Case 27. Malignant melanoma of prepuce. Melanin laden cells with short processes. × 650

16. Case 34. Malignant melanoma, scrotum. "Mosaic" patterning, with grouping of cells to resemble possible nerve-ending figures. c.f. fig. 32. × 650

17. Case 37. Bleached heavily-pigmented cells in malignant melanoma of scrotum, showing a typical nuclear pattern. × 650

18. Case 55. Benign cellular melanoma. The cells are closely packed, and show pale nuclei with prominent nucleolus. The cells stain poorly, and are variably pigmented. c.f. fig. 1. × 360

19. Case 43. Benign cellular melanoma. Variable pigmentation of cells, which tend to form groups, and some cells appear to be clasping others. × 360

20. Case 51. Benign cellular melanoma. Swirls of almost unpigmented cells in dense masses of heavily-pigmented cells. × 90

21. Case 63. Superficial zone of benign cellular melanoma, showing groups of cells (? nerve-ending-like figures) just below thinned surface epithelium. × 90

22. Case 82. A group of benign fibrous melanomas from the skin of one dog. Note the variable pigmentation of the cut surfaces. The black points are where hair-follicles are surrounded by pigmented tissue. Scale in cms.

23. Case 48. Benign cellular melanoma, growing up from depth of dermis, displacing hair follicles, and bulging upwards. × 5

24. Case 82. Patchily pigmented fibrous melanoma. × 2½

25. Case 71. Fibrous melanotic tumour, showing a number of pigmented cells in rather whorled fibrous tissue. × 260

26. Case 82. Whorled knotted fibrous structure of fibrous melanoma. × 90

27. Case 86. Base of melanotic fibroma from flank, showing a number of apparent origins of fibrous tissue from sheaths of small nerves. × 65

28. Case 86. Higher power view, showing small nerve near middle right margin of fig. 27. × 260

29. Case 82. Hyperplasia of surface epithelium, accumulation of melanotic cells below epithelium, and invasion of underlying fibrous tumour. × 90

30. Case 79. Pigmented cells in fibrous melanoma; some are long and narrow, some plumper, with short processes. × 260

31. Case 88. Plump cells from a fibrous melanoma. One appears to be clasping another. × 360

32. Case 80. Groups of cells in a fibrous melanoma, suggesting possible nerve ending figures. c.f. fig. 16. × 260

33. Case 77. Fibrous melanoma of prepuce. showing felted mass of dendritic melanoblasts. × 65

34. Case 77. Same tissue as fig. 33, under higher magnification. ×360

All sections stained haematoxylin and eosin.

# STUDIES ON ETHIDIUM BROMIDE

## VI. The Prophylactic Properties of the Drug

By

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### INTRODUCTION

Ethidium bromide (2, 7 diamino-10-ethyl-9-phenyl phenanthridinium bromide) has been shown to have marked curative properties when used against early *T. congolense* infections in cattle (Wilde and Robson, 1953; Wilson and Fairclough, 1953; Ford, Wilmshurst and Karib, 1953 b) and also against early *T. vivax* infections (Ford, Wilmshurst and Karib, 1953 a).

The usefulness of a purely curative drug is limited in the field by the fact that cattle in contact with tsetse flies are liable to reinfection and it would be a great advantage if the administration of a trypanocidal drug was followed by a period of protection against reinfection. On the other hand, prophylaxis produced by the slow release into the blood stream of relatively insoluble drugs has the disadvantage that the occurrence of low blood levels towards the end of the protective period may allow the infecting trypanosome to develop drug resistance. Therefore, for practical application, a reasonable period of complete protection is necessary.

### METHODS

The experiment was carried out at Malakal in the Southern Sudan, a district free of tsetse flies, but where transmission of trypanosomiasis by biting flies is known to occur. The plan was to dose one hundred cattle at each of two drug levels and, at varying intervals after this treatment, to challenge two groups of ten animals with *T. congolense* and *T. vivax* respectively. The drug levels chosen for the test were 1.0 mg./kg. the recommended curative dose, and 5.0 mg./kg. a dose which had not produced liver damage in a toxicity experiment (Ford, Wilmshurst and Karib, 1954). The drug in 1 per cent or 2 per cent aqueous solutions was injected subcutaneously on the side of the thorax above the elbow, using cattle of the Shorthorn zebu type.

The first challenge was carried out fourteen days after treatment. Ten animals from each drug group and ten untreated controls were challenged with *T. congolense* and parallel groups were challenged with *T. vivax*, making a total of sixty cattle for each challenge. Each animal was given 5 ml. of citrated blood from a donor animal whose blood was rich in trypanosomes.

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Commencing in the second or third week after the challenge, blood smears from all the animals in a group were examined on three consecutive days in each week. Examinations were continued for a total of sixteen weeks in the groups of bulls which had received ethidium bromide and the controls were examined until a high proportion of infections were detected. Then the controls were treated with either 1.0 or 2.0 mg./kg. of ethidium bromide, administered by intramuscular injection into the buttock.

The second challenge with *T. congolense* and *T. vivax* was carried out fourteen days after the first, again using ten cattle from each treatment group and ten controls for each trypanosome. The third challenge was carried out fourteen days after the second and the fourth fourteen days after the third. The preliminary results of the fourth challenge with *T. congolense* suggested that little drug protection against this trypanosome remained and so for the fifth challenge, carried out twenty-eight days after the fourth, only *T. vivax* was used to challenge ten animals treated at 1.0 mg./kg. and ten at 5.0 mg./kg. together with ten untreated controls.

## RESULTS

### *Local Reactions*

Following subcutaneous injection of the drug, local reactions, in the form of hard painful swellings, occurred below the site of injection and gravitated to the fold of skin at the elbow. The bulls grazed each day at some distance from the laboratory and received no supplementary food. In many cases a large necrosed area of skin developed in the fold of the elbow. This made movement difficult and was probably aggravated by walking. The animals lost condition and a number of deaths occurred, particularly in the 5.0 mg. kg. group where the tissue damage was most severe probably due to the greater amount of drug injected.

Because such severe reactions had followed the subcutaneous injections we decided that, when it became necessary, the control animals should be treated by the intramuscular route. Following these intramusuclar injections into the buttock there was a small swelling and slight lameness in some cases, but these symptoms disappeared leaving an occasional small scar at the site of injection. Thus the local reactions following intramuscular injection were negligible compared with those following subcutaneous injection and the intramuscular route has since been recommended as the most satisfactory method of administration.

### *Blood Examinations*

Tables 1 to 9 show the results of blood examinations following the various challenges.

#### *Protective action against T. congolense*

Tables 1, 2, 3 and 4 show that the strain of *T. congolense* used for the challenge was capable of producing a high infection rate in untreated cattle within three weeks after injection.

TABLE 1

FIRST CHALLENGE WITH *T. congolense* TWO WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 441 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 447 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 455 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 464 | — | — | — | — | — | — | — | — | | + | — | — | — | — | |
| | | 477 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 429 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 485 | — | — | — | — | died | | | | | | | | | | |
| | | 497 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 414 | + | + | + | + | + | + | + | died | | | | | | | |
| | | 396 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| B | 5.0 | 424 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 397 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 884 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 501 | — | — | — | — | died | | | | | | | | | | |
| | | 487 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 494 | — | — | — | — | — | — | — | — | died | | | | | | |
| | | 454 | — | — | — | — | — | — | died | | | | | | | | |
| | | 474 | — | — | — | — | — | — | — | — | | + | — | died | | | |
| | | 466 | — | — | — | — | — | — | — | — | | — | + | — | — | — | |
| | | 425 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| Controls | | 10 bulls | All 10 bulls were blood positive by the second week after challenge and were treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | | | |

\+ = one or more trypanosomes seen in thick blood films examined thrice weekly.

— = no trypanosomes seen at thrice weekly examinations.

The explanation of + and — applies also to tables 2 – 9.

TABLE 2

SECOND CHALLENGE WITH *T. congolense* FOUR WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 10 bulls | All blood negative for sixteen weeks. | | | | | | | | | | | | | |
| B | 5.0 | 394 | — | — | — | — | — | — | — | — | died | | | | | |
| | | 422 | — | — | — | died | | | | | | | | | | |
| | | 434 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 452 | — | — | — | — | — | — | — | — | — | — | died | | | |
| | | 473 | — | — | — | — | — | — | — | — | — | — | — | died | | |
| | | 484 | — | — | — | — | — | died | | | | | | | | |
| | | 492 | — | — | — | died | | | | | | | | | | |
| | | 500 | — | died | | | | | | | | | | | | |
| | | 868 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Controls | | 10 bulls | Eight bulls were blood positive by the third week and the whole group was treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | | |

TABLE 3

THIRD CHALLENGE WITH *T. congolense* SIX WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 393 | — | — | + | + | + | treated | | — | — | examinations ceased | | | | |
| | | 410 | — | + | + | + | + | | | | — | | | | | |
| | | 427 | + | + | + | + | + | | | | — | | | | | |
| | | 439 | — | — | — | — | — | | | | — | | | | | |
| | | 444 | — | — | + | + | + | | | | — | | | | | |
| | | 450 | — | + | + | + | + | | | | — | | | | | |
| | | 460 | — | — | — | — | — | | | | — | | | | | |
| | | 470 | — | + | + | + | | | | | — | | | | | |
| | | 481 | — | — | + | + | + | | | | — | | | | | |
| B | 5.0 | 392 | — | — | — | + | — | — | — | — | — | — | — | — | — | — |
| | | 419 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 453 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 451 | — | — | — | — | — | — | — | — | — | died | | | | |
| | | 446 | — | — | — | — | — | — | — | — | — | died | | | | |
| | | 472 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 483 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 491 | — | — | — | + | — | — | — | — | — | — | — | — | — | — |
| | | 499 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 860 | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Controls | | 10 bulls | All ten bulls were blood positive by the second week after challenge and were treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | | |

TABLE 4

FOURTH CHALLENGE WITH *T. congolense* EIGHT WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 403 | — | | + | + | treated and examinations ceased | | | | | | | | | |
| | | 425 | — | | + | + | | | | | | | | | | |
| | | 437 | + | | + | + | | | | | | | | | | |
| | | 442 | + | | + | + | | | | | | | | | | |
| | | 449 | + | | + | + | | | | | | | | | | |
| | | 459 | + | | + | + | | | | | | | | | | |
| | | 469 | + | | + | + | | | | | | | | | | |
| | | 488 | + | | + | + | | | | | | | | | | |
| | | 390 | + | | | | | | | | | | | | | |
| | | 479 | + | | | | | | | | | | | | | |
| B | 5.0 | 389 | — | | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 418 | + | | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 430 | — | | + | — | — | — | — | — | — | — | — | — | — | — |
| | | 438 | — | | — | — | — | — | — | — | — | — | — | — | — | + |
| | | 471 | + | | + | + | + | + | died | | | | | | | |
| | | 496 | — | | — | — | — | — | — | — | — | — | died | | | |
| | | 820 | — | | — | — | + | + | + | + | + | + | + | + | + | + |
| | | 836 | — | | — | — | + | + | + | + | + | + | + | + | + | + |
| | | 482 | — | died | | | | | | | | | | | | |
| | | 490 | — | | | | | | | | | | | | | |
| Controls | | 10 bulls | Seven bulls were blood positive by the third week and the whole group was treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | | |

Ethidium bromide at the rate of 5.0 mg./kg. gives a degree of protection up to six weeks after administration. Table 4 shows that by the eighth week there were three complete relapses out of ten animals challenged (bulls 471, 820 and 836), whilst three other animals (bulls 418, 430 and 438) showed a transient blood infection. Throughout the period there was a number of cases of transient infection, e.g. bull 466, twelve weeks after challenge, bulls 392 and 491 (table 3) six weeks after challenge and bull 438 (table 4) sixteen weeks after challenge. The significance of these results will be discussed later.

Ethidium bromide at the rate of 1.0 mg./kg. gives a degree of protection up to four weeks after administration. The first group showed one infected animal (bull 414, table 1) two weeks after challenge and following the six week challenge (table 3) seven out of nine bulls developed persistent infections. In these animals there was one transient infection (bull 464, table 1), which was blood positive eleven weeks after being challenged two weeks after drug treatment.

*Protective action against T. vivax*

This trypanosome produced a high infection rate in untreated controls on three occasions (tables 5, 6 and 7). When used in the two later challenges (tables 8 and 9) this result was not obtained and it is concluded that the inoculum was not infective, in spite of the fact that smears of the citrated blood had contained active trypanosomes before inoculation. Therefore the latest successful challenge with *T. vivax* was six weeks after drug treatment (table 7) and, up to this time, there was a degree of protection against the trypanosome. Table 5 shows that this protection was not complete.

## DISCUSSION

It is difficult to interpret the significance of some of the infections after the challenge dose. Several, such as those in Group A of table 2 and Group A of table 4, were persistent and occurred at the same interval after challenge as the infections in the controls. There is little doubt that these Group A infections were due to the failure of ethidium bromide at 1.0 mg./kg. to protect against the challenge strain of *T. congolense*. On the other hand, Group B results (tables 3 and 4) showed that the drug at 5.0 mg./kg. has a protective action up to six weeks, in that numerous persistent infections did not occur soon after the challenge, yet two bulls (392 and 491) showed a transient infection six weeks after the challenge and twelve weeks after drug administration.

It is possible that the drug does not protect completely, but rather allows the establishment of a suppressed infection which is only occasionally detected in the blood-stream. On the other hand,

TABLE 5

FIRST CHALLENGE WITH *T. vivax* TWO WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 8 | — | — | — | — | — | — | — | — | | — | — | — | — | | |
| | | 31 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 45 | — | — | — | — | — | — | — | — | | — | — | — | — | — | + |
| | | 72 | — | — | — | — | — | — | — | — | | — | — | — | — | + | — |
| | | 84 | — | — | — | — | — | — | — | — | | + | + | + | + | + | — |
| | | 110 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 341 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 352 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 365 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 378 | — | — | — | — | | — | — | — | | — | — | + | — | — | — |
| B | 5.0 | 24 | — | — | — | — | — | — | — | — | | died | | | | | |
| | | 65 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 75 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 88 | + | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 104 | — | — | — | — | — | — | — | — | died | | | | | | |
| | | 336 | — | — | — | — | — | — | — | — | | — | — | — | + | — | — |
| | | 347 | — | — | — | — | — | — | — | — | | — | — | — | — | — | — |
| | | 359 | — | — | — | — | — | — | — | — | | — | — | — | — | — | |
| | | 367 | — | — | — | — | — | — | — | — | | + | — | — | — | — | — |
| | | 385 | — | — | — | — | — | — | — | — | | — | — | — | + | + | |
| Controls | | 10 bulls | Nine bulls were blood positive by the second week after challenge and were treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | | | |

TABLE 6

SECOND CHALLENGE WITH *T. vivax* FOUR WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *No. of bulls used* | |
|---|---|---|---|
| A | 1.0 | 10 | All negative for 16 weeks |
| B | 5.0 | 9 | All negative for 16 weeks |
| Controls | | 10 | Eight bulls were blood positive by the third week and the whole group was treated with ethidium to reduce the risk of cross infection. |

TABLE 7

THIRD CHALLENGE WITH *T. vivax* SIX WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *No. of bulls used* | |
|---|---|---|---|
| A | 1.0 | 10 | All negatives for 16 weeks |
| B | 5.0 | 10 | All negative for 16 weeks |
| Controls | | 10 | Eight bulls were blood positive by the third week and the whole group was treated with ethidium to reduce the risk of cross infection. |

TABLE 8

FOURTH CHALLENGE WITH *T. vivax* EIGHT WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bull No.* | *Weeks after challenge* | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 17 | — | — | — | — | — | — | — | — | + | — | + | + | + |
| | | 33 | — | — | — | — | — | — | — | — | — | — | — | — | + |
| | | 61 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 78 | — | — | — | — | — | — | + | — | — | + | — | — | — |
| | | 94 | — | — | — | — | — | — | — | — | — | — | — | + | + |
| | | 335 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 345 | — | — | — | — | — | — | — | — | — | — | — | + | — |
| | | 370 | — | — | — | — | — | — | — | — | — | + | — | — | — |
| | | 355 | — | — | — | — | — | — | — | — | — | — | + | + | + |
| | | 3 | — | — | | | | | | | | | | | |
| B | 5.0 | 14 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 32 | — | — | — | — | — | | — | — | — | — | — | — | — |
| | | 81 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 67 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 97 | — | — | — | — | — | — | — | — | + | + | + | + | + |
| | | 106 | — | — | — | — | — | — | — | + | — | — | — | — | — |
| | | 340 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 351 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| | | 362 | — | — | — | — | — | died | | | | | | | |
| | | 371 | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Controls | | 10 bulls | One bull was blood positive by the third week and the whole group was treated with ethidium to reduce the risk of cross infection. | | | | | | | | | | | | |

TABLE 9

FIFTH CHALLENGE WITH *T. vivax* TWELVE WEEKS AFTER DRUG TREATMENT

| *Group* | *Dose mg./kg.* | *Bulls used* | *Weeks after challenge* | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| A | 1.0 | 10 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| B | 5.0 | 10 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| Controls | | 15 | — | — | — | — | — | — | | | | | | | | | |
| | | 37 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 100 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 268 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 274 | — | — | — | — | + | — | — | + | + | — | — | — | — | — | |
| | | 280 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 297 | + | — | — | — | — | + | + | — | — | — | + | — | — | — | |
| | | 407 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 421 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | |
| | | 856 | + | — | — | — | — | — | — | — | — | + | — | — | — | — | |

the biting-fly population of the Malakal district may produce cross-infection from either the infected controls or from trypanosome infections occurring in the cattle belonging to the local tribes and grazing near the experimental cattle. These cross-infections would conceivably be caused by fewer trypanosomes than the syringe infection used for the challenge and therefore could be transient in nature. The majority of these suspected biting fly infections appeared at least eleven weeks after drug administration, by which time the residual protection must have been very low or absent, as demonstrated by table 4 and this could account for the failure of the drug to protect against them. The only transient infection occurring earlier than eleven weeks after drug treatment was in bull 88 (table 5) at four weeks.

The occurrence of biting-fly-borne cross infection is strongly suggested in table 9, where transient infections occurred in the control group. It is unlikely that any of the infections in these animals were due to challenge trypanosomes, which failed to produce normal infection by the third week after injection and they could not have been due to suppression of the infection by ethidium as none had been administered to these controls.

More clear cut results might have followed if the experiment could have been carried out where biting flies were less frequent than in the Malakal area.

## CONCLUSIONS

The prophylactic action of a single dose of ethidium bromide has been assessed against a single massive syringe challenge of *T. congolense* and *T. vivax*. Against *T. congolense* 1.0 mg./kg. of ethidium bromide gives some protection for four weeks and 5.0 mg./kg. gives some protection for six weeks.

Against *T. vivax* both 5.0 and 1.0 mg./kg. of ethidium bromide give some protection up to six weeks and possibly longer, although the results at eight and twelve weeks were invalid, due to the failure of the challenge injection to infect the untreated controls.

A number of transient infections occurred in many of the groups and their significance is discussed.

## ACKNOWLEDGMENTS

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## REFERENCES

Ford, E. J. H., Wilmshurst, E. C., and Karib, A. A. (1953a). *Vet. Rec.*, **65**, 589; (1953b). *Ibid.*, **65**, 907; (1954). *Brit. vet. J.*, **110**, 96.
Wilde, J. K. H., and Robson, J. (1953). *vet. Rec.*, **65**, 49.
Wilson, S. G., and Fairclough, R. (1953). *Ibid.*, **65**, 201.

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# STUDIES ON *TRICHOSTRONGYLUS AXEI*

## III. The Course of Untreated Infestations

By

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### INTRODUCTION

During the course of the studies reported in parts I and II of the present series of papers (Gibson 1954a, b) it became obvious that sheep dying soon after infestation showed different lesions and clinical symptoms from those which survived for a longer period and it was therefore considered profitable to plan an investigation to trace the course of the disease in sheep caused by *T. axei*, beginning at the time of infestation and continuing until one year had elapsed. It was hoped to be able to correlate egg count and clinical symptoms with lesions and worm counts at different stages of the disease.

### MATERIALS AND METHODS

Eight Dorset Horn lambs, specially reared so as to be free from all intestinal parasites except *Strongyloides papillosus* and coccidia, were used in this experiment and when two months old each lamb was given 60,000 infective larvae of *T. axei* at the rate of 4,000 larvae per day. At the same time the collection of faecal samples began and continued four times weekly during the course of the experiment. The samples were examined for the presence of nematode eggs by the modified McMaster method or the salt flotation method according as the count was over or less than 100 eggs per gram. The animals were weighed weekly, care being taken to avoid weight changes due to the ingestion of large amounts of food or water just before weighing by preventing access to food and water for some twelve hours previously. The clinical condition of the animals was ascertained by daily inspection. Three animals died early in the experiment, one three weeks, one four weeks, and another five weeks after the end of the infection period. It was arranged that the others should be killed two months, three months, seven months, ten months and twelve months after the end of the infection period. All the animals were carefully examined post mortem and the lesions present carefully noted. In addition the number of worms present in the abomasum was estimated using the usual dilution method.

## RESULTS

A study of the results, summarised in Figs. 1 to 3 and Table 1, shows that the disease first has an acute phase during which the animal becomes extremely ill and during which it may die. In an animal which survives this phase the disease becomes less acute and finally becomes chronic during which phase the infestation may have little or no apparent effect on the animal.

TABLE 1

WORM COUNTS AND POST-MORTEM FINDINGS FOR THE EIGHT SHEEP

| *No. of lambs* | *Duration of infection in weeks* | *No. of T. axei found in abomasum at P.M.* | *Killed or died* | *Lesions found at P.M. examination* |
|---|---|---|---|---|
| 3108 | 3 weeks | 21,900 | D | Fair carcase but with no body fat. Severe abomasitis |
| 3107 | 4 „ | 26,500 | D | Poor carcase. Severe abomasitis |
| 3112 | 5 „ | 43,000 | D | „ „ „ |
| 3120 | 8 „ | 27,500 | K | Poor carcase. Slight abomasitis. A few commencing ulcers in abomasum |
| 3117 | 13 „ | 17,900 | K | Poor carcase. Many ulcers present in abomasum |
| 3094 | 28 „ | 1,450 | K | Good carcase. Abomasum had many ulcers with raised white edges and inflamed craters |
| 3095 | 40 „ | 250 | K | Good carcase. Many healed ulcers in abomasum |
| 3105 | 52 „ | 4,200 | K | Good carcase. Many healed ulcers in abomasum |

During the acute phase which lasts about six weeks, the three lambs, 3108, 3107 and 3112, died. The faecal egg count starts to increase as soon as the worms begin to mature and in sheep dying in this phase it rises steeply throughout the animal's life (Fig. 1). The animals continue to gain weight up to the time that the worms become mature when the lambs' weight gain is arrested and a loss of weight occurs. For example, Fig. 1 shows that lamb 3107 was seven pounds lighter at the time of its death than at the beginning of the experiment. A high worm burden was found to be characteristic of this phase of the disease, lambs 3108, 3107 and 3112 carrying respectively 21,900, 26,500 and 43,000 *T. axei* when examined post mortem. The carcases of these three lambs were extremely poor but post mortem examination revealed no noteworthy lesion except in an acute inflammation of the abomasum.

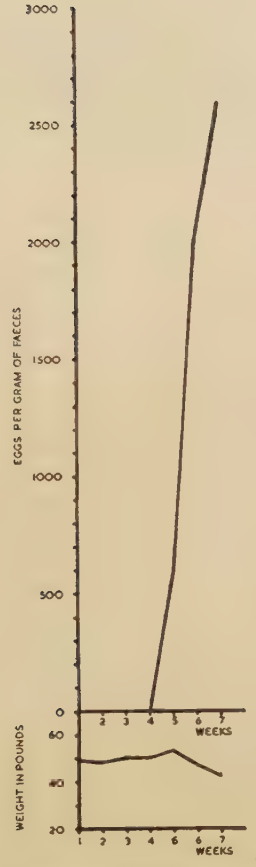


FIG. I SHOWING THE VARIATIONS IN FAECAL EGG COUNT AND WEIGHT GAIN OF LAMB 3107

The next phase of the disease is transitory and is typified by lambs 3120 and 3117. Lamb 3120 was slaughtered at the beginning of this phase and its egg count curve closely resembled that described for the acute phase. The weight curve, however, differed in that a period of weight loss, beginning when the worms reached maturity, was followed later by a period during which the animal began to gain weight again. At post-mortem examination the worm burden was quite high, 27,500 *T. axei* being present in the abomasum, but the abomasal lesions differed from those previously seen. The severe abomasitis described above had regressed and only a slight inflamation was present, but at a number of places the mucous membrane had been eroded to form round ulcers varying in size from an eighth of an inch up to as much as three quarters of an

inch in diameter. The carcase of the lamb was extremely poor and showed no trace of body fat. In lamb 3117 (Fig. 2) these processes had been taken a stage further. After an initial rise, the egg count showed a tendency to fall and the weight curve was similar to that

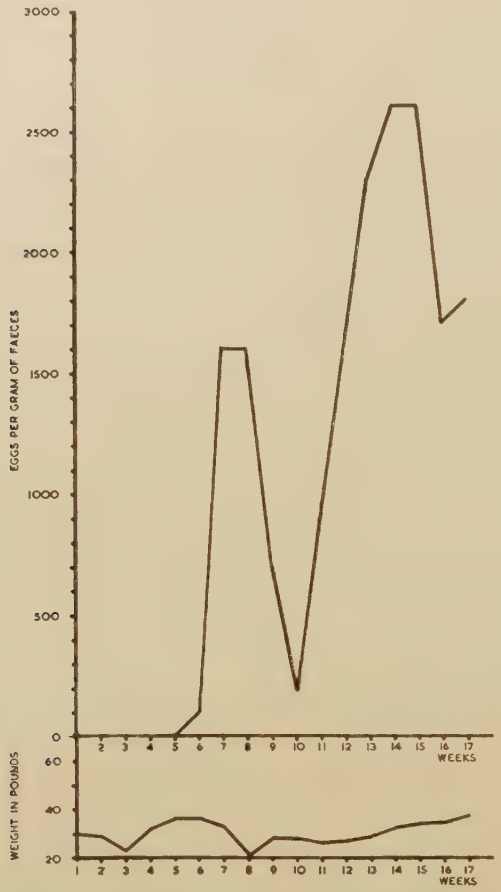


FIG. 2 SHOWING THE VARIATIONS IN FAECAL EGG COUNT AND WEIGHT GAIN OF LAMB 3117.

of 3120. At post mortem examination the carcase was poor and 17,900 *T. axei* were present in the abomasum. Ulcers similar to those found in lamb 3120, were seen in the abomasum but were present in much larger numbers than in this latter lamb.

The third stage of the disease process seen in lambs 3094, 3095 and 3105 is the chronic or recovery phase. In these lambs the egg count after an initial rise to a high level, fell again and by the twenty-fourth week of the experiment was very low indeed and remained low until the end of the experiment. The lambs suffered

an initial check in weight gain as a result of the worm infestation, but after the egg count began to decline the animals began to put on weight again and continued to do so until the end of the experiment. These effects are illustrated in Fig. 3 which records the data concerning lamb 3105. The worm burdens of the three lambs

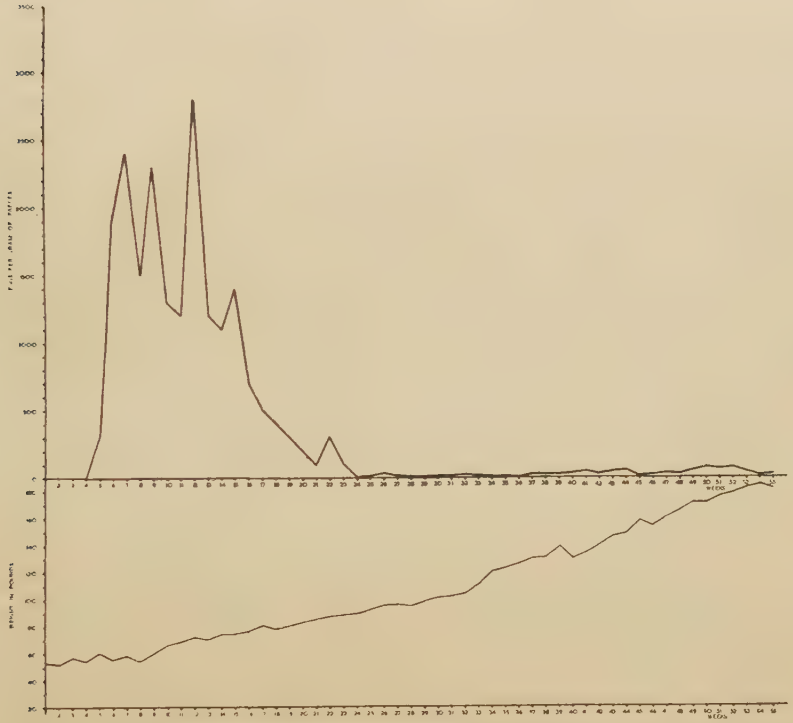

FIG 3 SHOWING THE VARIATIONS IN FAECAL EGG COUNT AND WEIGHT GAIN OF LAMB 3105

killed during the course of this phase were low, 1450, 250 and 4200 respectively for the three lambs 3094, 3095 and 3105. At post mortem examination the carcases of these lambs were found to be good, amply clothed with body fat, but there was a slight difference in the abomasal lesions of the three lambs. Generalised abomasal inflamation was not observed in any of the lambs but in 3094 there were distributed over the mucous membrane numerous crater-like ulcers with raised white edges, which looked very much as though circular plugs of mucous membrane had been removed using a cork borer. In 3095 the picture was similar except that the ulcers were healing and in 3105 the process had gone still further, the "craters" of the ulcers being covered with cicatricial tissue. These ringworm-like lesions are typical of the chronic stage of *T. axei* infestation.

## DISCUSSION

From this investigation it is clear that *T. axei* affects young sheep most severely and that as they become older they can throw off the infestation. Young lambs have clearly little or no resistance and, the reaction in such animals being acute, they may die in this stage. Animals which survive the acute stage pass through a transitory phase to a chronic or recovery phase when they gain weight rapidly and resemble normal sheep.

The lesions described in this paper appear not to have been previously described in lambs; in particular the ringworm-like lesions of the chronic phase seem to have escaped notice. Roth and Christensen (1942) give a very good description of two types of lesion caused by *T. axei* in horses. The first consists of circumscribed thickenings of mucosa—the hypertrophic processes, whilst the second are ringworm-like lesions similar to those described above in sheep. These two types of lesions have been noted by other authors in horses; for example Le Roux (1924) remarks that *T. axei* is found in horses in both ringworm-like lesions and in hypertrophic gastritis. The hypertrophic lesion has not been seen in any of the sheep used in the present experiment, nor in any of the other sheep artificially infected with *T. axei* by the writer so it seems likely that it does not occur. The ringworm-like lesion appears to be similar, however, in both species.

## CONCLUSIONS

An experiment designed to trace the course of untreated infestation of *T. axei* in sheep is described. The experiment showed that the disease has initially an acute phase characterised by a steeply rising faecal egg count, a rapid loss in weight and a severe abomasitis. This is followed by a transitory phase during which the faecal egg count begins to fall and during which the animal begins to put on weight again. Finally during the chronic or recovery phase the animal's egg count is low and it resumes an apparently normal rate of weight gain. At post-mortem examination during this latter phase, ringworm-like lesions are seen in the abomasum.

## REFERENCES

Gibson, T. E. (1954a). *J. comp. Path.*, **64**, 127; (1954b). *Ibid.*, 360.
Le Roux, P. L. (1924). *J. Helminthol.*, **2**, 111.
Roth, M., and Christensen, N. O. (1942). *Skand. vet. Tidsk.* **20**, 488.

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# STUDIES ON HOST RESISTANCE TO DICTYOCAULUS INFECTION

## I. The Phenomenon of Inhibited Development

By

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### INTRODUCTION

The phenomenon of retarded or inhibited development of nematode parasites has long been recognised, but recently interest in it has been renewed by the observations of Kotlan (1949) and by the findings of Gibson (1953) who showed that immature *Trichonema* larvae can persist for prolonged periods in the intestinal wall of the horse. Observation concerning the occurrence of the phenomenon in infections of *Trichostrongylus retortaeformis* (Michel, 1952) and those of Somerville (1953) with *Ostertagia circumcincta* might also be mentioned. The phenomenon has been discussed in general terms (Taylor and Michel, 1953) and a preliminary note on its occurrence in *Dictyocaulus* infections has also appeared (Taylor and Michel, 1952). It is the purpose of the present paper to present the evidence establishing the occurrence of inhibition of development of *Dictyocaulus viviparus* and demonstrating its connection with host resistance. Since these observations have aroused some interest in connection with parasitic bronchitis of the adult bovine and with fog fever, the significance of immature worms in the lungs of cattle will be discussed, as will the relevance of our findings to the epidemiology of husk.

### EARLY FIELD EVIDENCE

Up to 1950, acute parasitic bronchitis of the adult bovine was often not recognised as such, since it was not revealed by the normal methods of diagnosis or of post-mortem examination. Observations by Taylor (1935) and experimental work on *D. filaria* infestations in sheep suggested that perhaps immature worms might be responsible and consequently a search for these was made in such cases as came to hand. In the first of these the lungs contained some six hundred immature worms, all less than 3 mm. long. The affected animals in this outbreak were housed and a month later a further two animals were slaughtered for post-mortem examination. From the lungs of one of these animals, ten immature worms, all less than 1 cm. long, were recovered. From those of the other, approximately fifty immature worms less than 3 mm. long were recovered.

From the lungs of an animal in another outbreak which had also been housed for a month, 457 worms less than 3 mm. long were

recovered. It appeared, therefore, not only that immature forms were present in these cases, but also that their rate of growth must have been greatly retarded. It was presumed that these immature forms must in some way be connected with the occurrence of symptoms of disease, perhaps even with the fog-fever-like syndrome which was at that time regarded as a manifestation of an atypical form of parasitic bronchitis. Accordingly, an opportunity was sought to determine whether this retarding or inhibiting of development occurred in animals in which symptoms of disease were not present.

Two calves that had been turned out on the 24th April, 1950, on a small paddock and which had subsequently proved to be infected with lungworms were brought indoors on 13th October, 1950 and maintained under such conditions of cleanliness as would preclude the possibility of infection. One of these was shot on the 10th November, 1950, twenty-eight days after being removed from a source of infection, and its lungs examined. One hundred and sixty-one worms ranging in length from 1 mm. to 1 cm. were recovered, together with two others approximately 2 cm. long.

The other animal was killed on the 27th December, seventy-five days after being removed from a possible source of infection. From the lungs of this animal 164 worms, less than 3 mm. in length, were recovered, together with 37 less than 1 cm., 12 less than 2 cm. and four mature adults.

## EXPERIMENTAL INFECTIONS

### *Administration of Larvae to Resistant Animals*

Fourteen animals that had recovered from experimental infections of *D. viviparus* were reinfected with large single doses of larvae and killed at intervals ranging from 24 to 279 days after reinfection. Particulars of these animals are given in Table 1. Their faecal larval counts are shown in Fig. 1, from which it will be seen that in no case did the second infection become patent. The precise fate of larvae administered to an animal that has recovered from a previous infection is still under investigation and will be the subject of another paper. Table 1 shows, however, that half of the animals slaughtered were found to be harbouring immature lungworms even though kept in such a manner as to preclude the possibility of accidental infection. The actual numbers recovered were not large and diminished with increasing lengths of time after reinfection, but it is clear that immature forms are capable of persisting for long periods of time.

### *Comparison of Rates of Development in Resistant and Susceptible Animals*

That this inhibition of development is connected with the resistance of the host or depends upon previous experience of infection

TABLE 1

REINFECTED ANIMALS

Killed after intervals of time greater than the minimum prepatent period

| *No.* | *Date first infected* | *Dose* | *Date re-infected* | *Dose* | *Days since first infected* | *Date killed* | *Days since last infection* | *Worms recovered post-mortem* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *>3 cm.* | *>2 cm.* | *>1 cm.* | *>3 mm.* | *<3 mm.* |
| 2146 | 14.3.52 | 25,000 D | 16. 9.52 | 200,000 | 186 | 10.10.52 | 24 | — | — | — | — | 331 |
| 2163 | 7.4.52 | 35,000 D | 21.10.52 | 200,000 | 197 | 18.11.52 | 28 | — | — | — | — | — |
| 2194 | 24.4.52 | 10,000 | 27.3.53 | 100,000 | 336 | 27. 4.53 | 31 | — | — | — | 3 | 47 |
| 2129 | 25.1.52 | 5,000 | 18.8.52 | 200,000 | 205 | 4.10.52 | 57 | — | — | — | 2 | 16 |
| 1874 | 1.12.50 | 50,000 | 1.3.51 | 50,000 | 90 | 30. 4.51 | 61 | — | — | — | 12 | 156 |
| 2188 | 19.4.52 | 5,000 | 27.3.53 | 100,000 | 342 | 27. 5.53 | 61 | — | — | — | — | — |
| 2128 | 25.1.52 | 10,000 | 7.8.52 | 400,000 | 194 | 14.10.52 | 68 | — | — | — | — | — |
| 1886 | 25.2.51 | 10,000 | 5.6.51 | 100,000 | 100 | 5. 9.51 | 91 | — | — | — | 1 | 13 |
| 2082 | 29.10.51 | 5,000 | 16.9.52 | 200,000 | 322 | 19. 2.53 | 113 | — | — | — | — | — |
| 1907 | 7.4.51 | 50,000 | 14.8.51 | 100,000 | 129 | 21. 1.52 | 160 | — | — | — | — | — |
| 1908 | 7.4.51 | 25,000 | 14.8.51 | 100,000 | 129 | 22. 1.52 | 161 | — | — | 11 | 8 | 14 |
| 2073 | 26.9.51 | 25,000 | 7.5.52 | 200,000 | 223 | 10.10.52 | 177 | — | — | — | — | — |
| 2081 | 29.10.51 | 50,000 | 29.5.52 | 200,000 | 212 | 2. 1.53 | 215 | — | — | 1 | 2 | 2 |
| 2083 | 29.10.51 | 25,000 D | 7.5.52 | 200,000 | 200 | 20. 2.53 | 279 | — | — | — | — | — |

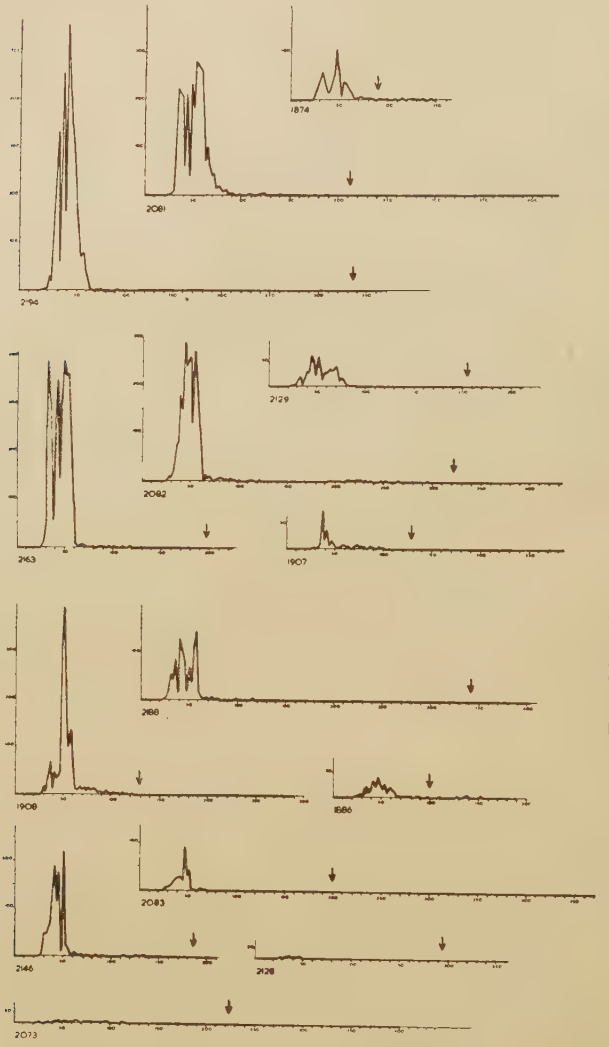



FIGURE 1

Faecal larval counts of reinfected calves.
The first dose of larvae was given on day O, the second on the day indicated by the vertical arrows.

was demonstrated by comparisons between the size of worms from resistant and susceptible calves. Four pairs of calves of equal age were selected, a dose of larvae being administered to one of each pair. After two or three months a larger dose of larvae was administered to both calves which were killed 8 to 12 days later. One hundred worms from each calf, recovered by means of the Baermann apparatus were measured. In each case the worms from the calf that had had previous experience of infection were smaller than those from the susceptible calf and the difference proved to be statistically significant. Details are set out in Table 2.

TABLE 2

DIFFERENCE IN LENGTH OF WORMS

Details of four pairs of calves, showing that worms recovered eight or twelve days after the administration of larvae are larger in susceptible than in previously infected animals. The significance of the difference between means was determined by a "t" test which gave a value for p of less than 0.001 in every case.

| *Pair No.* | *Calf No.* | *Date infected* | *Dose* | *Date infected* | *Dose* | *Date killed* | *Days since infection* | *Mean of lengths of 100 worms(μ)* |
|---|---|---|---|---|---|---|---|---|
| 1 | 2650 | 1. 8.53 | 5,000 | 24.10.53 | 100,000 | 5.11.53 | 12 | 767.42 |
| | 2657 | | | 24.10.53 | 100.000 | 5.11.53 | 12 | 1190.91 |
| 2 | 2579 | 27. 5.53 | 500 | 8. 7.53 | 10,000 | 20. 7.53 | 12 | 1322.29 |
| | 2592 | | | 8. 7.53 | 10,000 | 20. 7.53 | 12 | 1547.47 |
| 3 | 2810 | 24.11.53 | 4,500 | 10. 2.54 | 40,000 | 22. 2.54 | 12 | 773.19 |
| | 2827 | | | 10. 2.54 | 40,000 | 22. 2.54 | 12 | 1581.14 |
| 4 | 2826 | 24.11.53 | 4,500 | 10. 2.54 | 40,000 | 18. 2.54 | 8 | 622.18 |
| | 2849 | | | 10. 2.54 | 40,000 | 18. 2.54 | 8 | 1034.91 |

## *Administration of Larvae in Twenty Daily Doses*

In the course of our experimental work a number of animals were infected, not by means of a single dose of larvae but by twenty daily doses. Six of these animals died and details of the helminthological findings are given in Table 3. It will be seen that a proportion of the worms failed to develop at the normal rate in every case. If the inhibition of development is to be regarded as a phenomenon

TABLE 3

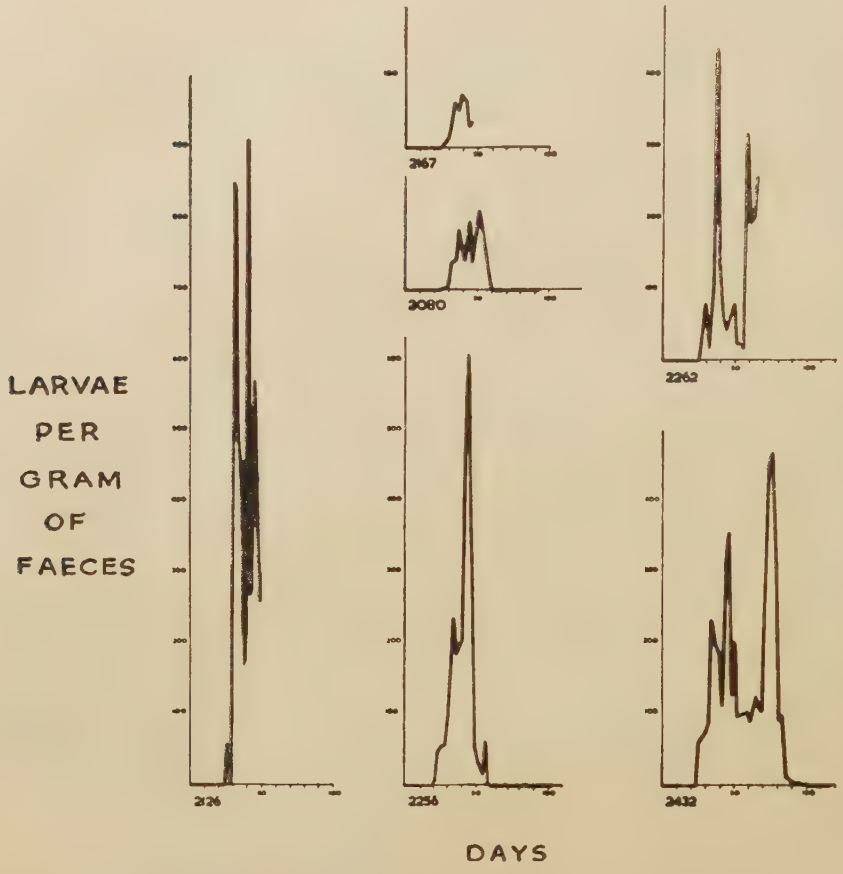
DAILY DOSES OF LARVAE

Details of animals that died at various times after receiving 20 equal daily doses of larvae.

| *No.* | *Date infection began* | *Total of larvae administered* | *Date of death* | *Days since last infection* | *Worms recovered post-mortem* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *3 cm.* | *2 cm.* | *1 cm.* | *3 mm.* | *3 mm.* |
| 2167 | 7. 4.52 | 10,000 | 25. 5.52 | 28 | 87 | 83 | 13 | 15 | 15 |
| 2126 | 18. 1.52 | 35,000 | 9. 3.52 | 33 | 2527 | 1644 | 735 | 191 | 1000 |
| 2262 | 7. 7.52 | 10,000 | 10. 9.52 | 45 | 363 | — | 1 | 29 | 30 |
| 2080 | 29.10.51 | 35,000 | 30. 1.52 | 73 | — | 27 | 47 | 7 | — |
| 2256 | 25. 6.52 | 10,000 | 7.10.52 | 84 | — | — | 3 | 50 | 10 |
| 2432 | 6. 2.53 | 10,000 | 4. 6.53 | 98 | — | 6 | 11 | 41 | 5 |

of resistance, then one must postulate that it is the later worms to be administered that do not develop at the normal rate. Reference to Fig. 2, in which the faecal larval counts of these animals are given, as well as to Table 3, will show that the inhibited forms tend

FIGURE 2



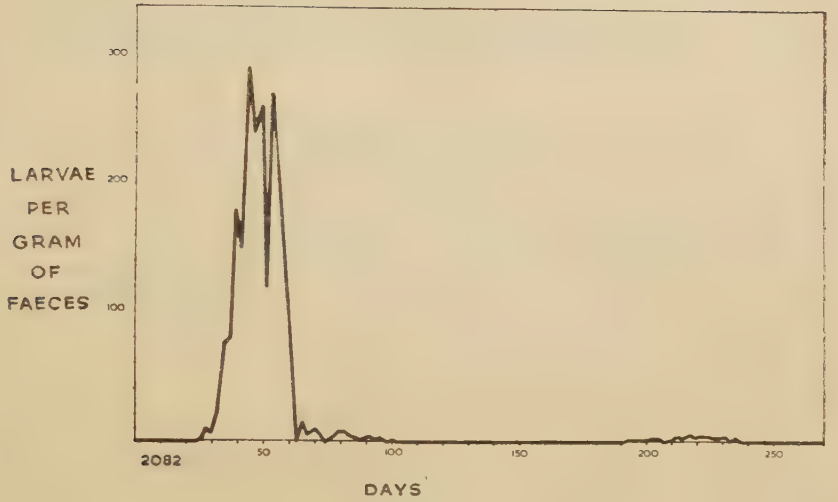

Faecal larval counts of calves given equal daily doses of larvae on days 0 - 19.

to persist even after the adult worm infection has been spontaneously terminated. Of those animals infected in this way and which did not die, one (2082) began to pass larvae again in its faeces more than ninety days after the infection appeared to have been completely eliminated. The faecal egg counts of this calf are shown in Fig. 3. It is concluded from this that the inhibited worms are capable of ultimately resuming their development.

*The Administration of a Single Dose of Larvae to Susceptible Animals*

Table 4 gives details of calves which, having had no previous experience of infection, received a single dose of larvae and died more than twenty days after. Here again a small proportion of the worms failed to develop and tended to persist after the infection had been terminated (see also Fig. 4). If it is indeed the later arrival

FIGURE 3




Faecal larval counts of calf 2082.

TABLE 4

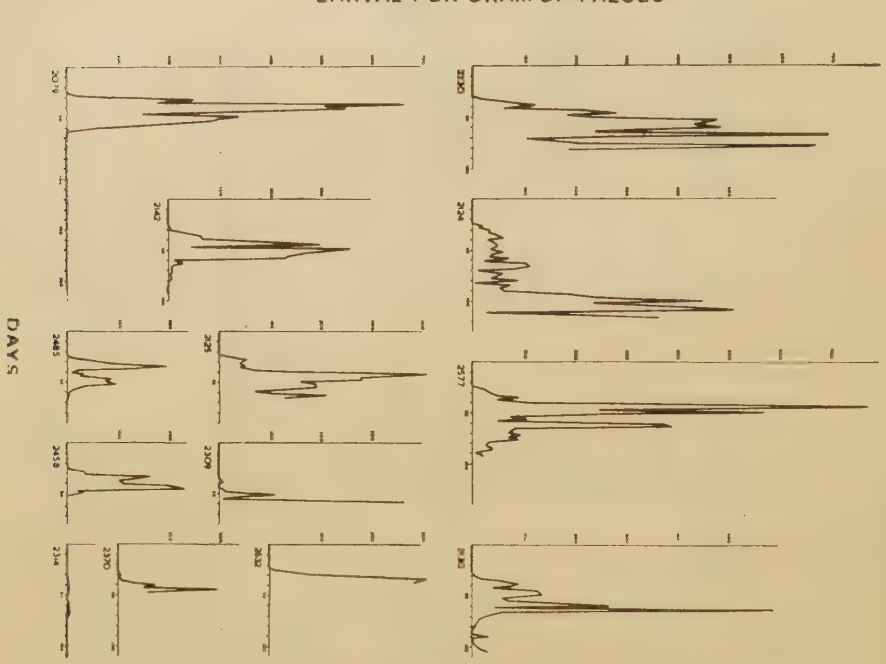
ADMINISTRATION OF SINGLE DOSE

Animals that died at various times after the administration of a single dose of larvae.

| *No.* | *Date infected* | *Dose* | *Date of death* | *Days after infection* | *Worms recovered post-mortem* | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | > *3 cm.* | > *2 cm.* | > *1 cm.* | > *3 mm.* | < *3 mm.* |
| 2222 | 17. 5.52 | 25,000 | 6. 6.52 | 20 | — | 904 | 6200 | 1 | 250 |
| 2132 | 29. 1.52 | 50,000 | 20. 2.52 | 22 | — | 1000 | 2600 | 2065 | 560 |
| 2608 | 2. 7.53 | 7,000 | 25. 7.53 | 23 | 466 | 499 | 170 | 35 | 12 |
| 2631 | 1. 8.53 | 5,000 | 24. 8.53 | 23 | 725 | 89 | 106 | — | 15 |
| 2430 | 6. 2.52 | 7,000 | 2. 3.53 | 24 | 185 | 133 | 78 | 18 | 13 |
| 2666 | 1. 8.53 | 5,000 | 26. 8.53 | 25 | 1423 | 748 | 306 | — | — |
| 2235 | 29. 5.52 | 10,000 | 23. 6.52 | 25 | 808 | 1578 | 104 | — | 80 |
| 2663 | 1. 8.53 | 5,000 | 28. 8.53 | 27 | 2070 | 85 | 45 | 3 | 18 |
| 2263 | 22. 7.52 | 10,000 | 19. 8.52 | 28 | 790 | 46 | — | — | — |
| 2223 | 17. 5.52 | 10,000 | 14. 6.52 | 28 | 991 | 737 | 73 | — | 57 |
| 2195 | 24. 4.52 | 5,000 | 22. 5.52 | 28 | 234 | 304 | 47 | — | 12 |
| 2661 | 1. 8.53 | 5,000 | 29. 8.53 | 28 | 317 | 39 | 52 | 27 | 14 |
| 2283 | 18. 8.52 | 5,000 | 16. 9.52 | 29 | 361 | 250 | 20 | — | — |
| 2145 | 14. 3.52 | 50,000 | 22. 4.52 | 39 | 2107 | 92 | 183 | 107 | — |
| 2632 | 1. 8.53 | 5,000 | 14. 9.53 | 45 | 1553 | 15 | 12 | — | 30 |
| 2370 | 24.12.52 | 25,000 | 11. 2.53 | 49 | 3320 | 91 | 4 | 22 | 60 |
| 2458 | 6. 2.53 | 7,000 | 1.4.53 | 55 | — | — | 2 | — | 13 |
| 2309 | 11.10.52 | 150,000 | 10.12.52 | 62 | 2049 | 46 | 49 | 20 | 31 |
| 2125 | 18. 1.52 | 25,000 | 23. 3.52 | 65 | 224 | 76 | 83 | 7 | — |
| 2230 | 23. 5.52 | 10,000 | 13. 8.52 | 82 | 178 | — | — | — | — |
| 2485 | 6. 3.53 | 5,000 | 3. 6.53 | 89 | — | — | — | — | 8 |
| 2142 | 10. 3.52 | 5,000 | 12. 6.52 | 94 | — | — | — | 6 | 1 |
| 2577 | 27. 5.53 | 7,000 | 1. 9.53 | 96 | 46 | — | 5 | 6 | 3 |
| 2130 | 25. 1.52 | 25,000 | 11. 5.52 | 105 | 6 | — | 3 | — | — |
| 2314 | 31.10.52 | 200,000 | 20. 2.53 | 112 | — | — | — | — | 1 |
| 2124 | 18. 1.52 | 35,000 | 16. 5.52 | 119 | 317 | 93 | 49 | — | — |
| 2079 | 29.10.51 | 35,000 | 6. 6.52 | 220 | — | — | — | — | — |

in the lungs that do not develop, then the power to inhibit them must be very speedily acquired by the calf, since the arrival of the first is not separated from that of the last by more than a very few days.

FIGURE 4




Faecal larval counts of calves receiving a single dose of larvae only.

## DISCUSSION

It appears that even when a fully susceptible animal is given a single dose of *Dictyocaulus viviparus* larvae, the development of some of them is inhibited. We have observed a similar phenomenon in infections of *Trichostrongylus retortaeformis* and Sommerville recorded that *Ostertagia circumcincta* persisted for long periods in the gastric mucosa of sheep in similar circumstances. In spite of this, however, one must regard the inhibition of development as a phenomenon of resistance since prior experience of infection clearly affects the rate of development. In *T. retortaeformis* infections also, a much greater proportion of the larvae are inhibited in the resistant than in the susceptible animal.

Whether this mechanism of resistance plays any material part in protecting a calf from lungworm infection is questionable. The indications are that an infection administered to an animal that has recovered from a previous one does not become patent, because

the majority of the larvae fail to become established in the lungs and many of those that do, are eliminated after a short time. Those that persist fail to develop, but it is doubtful whether the effect of this in protecting the host is significant.

It is probably incorrect to think of immature inhibited forms as having an enhanced pathogenicity. The worm burdens of adult cattle that die of parasitic bronchitis are variable both in number and size distribution. In an investigation of the occurrence of the fog-fever syndrome in parasitic bronchitis (Michel, 1954) it was concluded that the extreme symptoms were associated with the process whereby the worms are eliminated. Where only small numbers of immature worms are recovered at post-mortem from the lungs of adult cattle affected with husk, they are likely to be the residue left over from the elimination of a larger number, and it is unwarrantable to assume that the few worms which are recovered had themselves given rise to the symptoms.

If the effect of the phenomenon as a defensive mechanism is slight and its relevance to pathology indirect, that to epidemiology may be more significant. Immature worms persist for long periods in the lungs of animals that have recovered from lungworm infections, but are capable of resuming their development. It follows that any animal that has been exposed to infection is a potential carrier of infection and since the output of larvae in the faeces may be intermittent, such carriers cannot be detected. That silent carriers play an important part in the epidemiology of husk emerges from field investigations, but we have never encountered carriers of the type described by Wetzel (1950) which, having contracted the infection one season, continued to excrete larvae through the winter and on into the next. In our experience, the course of naturally acquired lungworm infections is terminated in a very similar manner to that of experimental infections.

The examination of material from the field suggests that very few members of a group of animals that suffered from husk in the previous season, are passing larvae at any given time, and that the numbers of larvae they pass are small. Even small numbers of larvae may be highly significant, however, and we have been able to show that under favourable conditions a very rapid build up of infection on the pasture may occur.

## CONCLUSIONS

Adult bovines suffering from acute parasitic bronchitis have been found to harbour immature lungworms even when they have been housed for a month before death.

Immature worms can be recovered from animals that have been housed for a month or more, even when these animals have not shown any symptoms of parasitic bronchitis.

When calves that have recovered from an experimental lungworm infection are reinfected, this second infection does not become patent, but immature worms may persist in the lungs for some months.

The rate of growth of worms administered to calves that have had prior experience of infection is less rapid that that of worms administered to susceptible control calves.

When susceptible calves are given either a single dose or twenty daily doses of larvae a proportion of the larvae remain undeveloped.

Some of these inhibited forms may persist after the adult worms have been eliminated; they are, however, capable of resuming their development.

ACKNOWLEDGMENTS

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REFERENCES

Gibson, T. E. (1953). *J. Helminth.*, **27**, 29.
Kotlan, A. (1940). Rep. XIVth Int. vet. Cong., **2**, 61.
Michel, J. F. (1952). *Nature*, **169**, 933; (1954). *Vet. Rec.*, **66**, 381.
Sommerville. (1953). *Aust. J. agr. Res.*, **5**, 130.
Taylor, E. L. (1935). *Vet. Rec.*, **15**, 1280.
Taylor, E. L., and Michel, J. F. (1952). *Nature*, **169**, 753; (1953). *J. Helminth.*, **27**, 199.
Wetzel, R. (1950). *Tierärztl. Umsch.*, 235.

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# PRIMARY PULMONARY CARCINOMAS OF THE DOG

By

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## INTRODUCTION

The problem of primary pulmonary carcinoma has been widely discussed in human medicine and it is unanimously agreed that the incidence of lung cancer is increasing. In veterinary medicine the relevant literature is very scarce and gives the impression that this is a rare malignancy in domestic animals. Some of the recorded observations on lung tumours in dogs are very old and it is thus impossible for us to verify exactly which type of neoplasm they refer to.

Monlux (1952), in his valuable paper, collected most of the cases published in the literature and added 19 of his own; readers are referred to this publication for a detailed list of references. Although Monlux classified tumours morphologically, he did not attempt to deal with their histogenesis. In many of the older papers quoted by him, no information was given beyond the diagnosis and he himself points out how doubtful these records are. A number of other papers refer to carcinomas of the lung without giving details as to the neoplastic type, and in other more detailed reports, there seems to be a certain diversity of opinion as to the classification and the tissue of origin of the tumours.

Siegert (1893) and Tabusso (cited by Chierici, 1911) observed squamous cell carcinomas, which they think originated from alveolar epithelium which had undergone metaplasia. Monlux (1952) also saw a squamous cell carcinoma but he does not discuss its origin. Rievel (1906) and Drieux and Thiery (1945) reported cases of mucinous carcinomas, but whereas Rievel insists on an origin from the alveolar epithelium, the latter authors believe the peri-bronchial glands to be the parent tissue. In three of Monlux's cases (6, 13 and 14) mucin was found in the cells but he does not categorically state his opinion as to the origin of these neoplasms. Ball (cited by Giannini, 1948) and Chierici (1911) reported solid carcinomas which they thought to be derived from the alveolar epithelium. The case that Jackson (1936) diagnosed as an adeno-acanthoma was supposed to be derived from the pulmonary parenchyma. Schlotthauer and Millar (1951) referred to an alveolar adeno-carcinoma associated with hypertrophic osteo-arthropathy without giving details of the histogenesis of the pulmonary neoplasm. Authors who have described columnar cell carcinomas agree that they arise from the bronchi.

We can thus infer from the literature that primary lung carcinoma is very rare in the dog, and in fact not many more than 50 accurately diagnosed cases have been reported up to the present time. As no cases have been reported from Brazil, the authors

decided to collect the cases in the archives of the Department of Pathological Anatomy of the Faculty of Veterinary Medicine, São Paulo University.

## CASE REPORTS

*Case* 1

A ten-year-old male Alsatian dog was examined post-mortem.

*Macroscopic appearance.* Examination of the lungs revealed a firm rounded lesion about 2.5 cm. in diameter in the central portion of the left diaphragmatic lobe, located deep in the pulmonary parenchyma. The cut surface was yellowish-white in colour and the edges were well defined. In the right cardiac lobe there was a nodule presenting the same features, measuring about 0.7 cm. in diameter and also deeply located in the parenchyma.

In the brain there was a rather soft, rounded tumour, about 3 cm. in diameter, whitish in colour, situated in the right lateral lobe of the cerebellum near the peduncles. Extending beyond the tentorium cerebelli, it reached over the posterior part of the cerebral hemisphere where it formed a cavity between the ectosagittal fissure and the pyriform lobe. This lesion was covered by the moderately thickened dura mater.

The temporo-mandibular and masseter muscles on the same side had undergone atrophy.

*Histological appearance.* In the lungs, the growth consisted of multiple nodules, lying deep in the pulmonary parenchyma, separated from the latter only by a zone of compressed lung tissue, where the alveoli were either collapsed or, as in a few fields, filled with red blood cells.

The cells forming these nodules were of the columnar type, disposed in one or sometimes more layers. They formed solid buds with a radiating arrangement; a central lumen was present in some fields, a core of vascular connective tissue in others. Their growth appeared to imitate that of the bronchioles (Fig. 1). There was a little cytoplasm with discrete basophilia. In some cells a condensation of the upper edge forming a cuticle could be seen. No mucinous or ciliated cells were found. The nuclei were mostly elongated, though variable in shape and size. The chromatin was dense in some and loose in others. Most of the nuclei had one or two nucleoli. In many fields there were some cuboidal cells of varying size with rounded nuclei, the chromatin being either dense or loose. Apart from the papillary lesions, solid structures, made of round cells sometimes strongly resembling lymphocytes, sometimes more like polymorphonuclears, were seen. In addition, a great number of columnar, spindle-shaped or round cells appeared scattered throughout the connective tissue. The stroma was delicate and not abundant, consisting of vascular connective tissue. By silver impregnation methods reticular fibres were demonstrated in the stroma but there were none between the tumour cells.

Sections of the tumour in the brain showed a very similar structure. At some points the dura mater presented a proliferation resembling a lamellar meningioma. In many fields hyalinization of the connective tissue had occurred and quite often calcium salts were deposited.

*Diagnosis.* Columnar cell bronchogenic carcinoma.

*Case* 2

An eight-year-old female dog of no definite breed was examined post-mortem.

*Macroscopic appearance.* The lungs were of a pinkish red colour with anthracotic spots all over the surface. The consistency was soft and there were irregular areas with a moist and shiny appearance that would retain a digital impression. When cut, several areas of rather grayish colour were seen.

The thyroid glands were enlarged and soft. The cut surface showed the typical appearance of colloid goitre. The liver was congested and showed some necrotic foci. Both adrenal glands had a hypertrophied cortex and some cysts were present in the ovaries.

*Histological appearance.* The appearance of the sections varied greatly. Some parts were normal, in others there was an alveolar oedema, in still others the alveoli were filled with large polyhedral or rounded cells with slightly basophil cytoplasm; between these cells there were some neutrophils and plasmocytes. Other alveoli were lined with these cells (Fig. 2). In one section the wall of a bronchus was found covered by a stratified epithelium of polyhedral cells, the superficial layer being of squamous cells (Fig. 3). There were no tonofibrils between these cells. The basal membrane was interrupted at some points and the cells of the bronchial wall infiltrated the lamina propria. High magnification showed that the nuclei of the tumour cells were rounded, elongated or distorted, and of various sizes, with loose or dense chromatin with at times a blurred appearance. Some cells had 2 or 3 nuclei. One nucleolus was present in most of the tumour cells but some had more than one. Mitosis was frequently seen. There was no proper tumour stroma, this being represented by the pulmonary tissue. Silver impregnation failed to show reticulin between the neoplastic cells. The lesions did not form definite tumour nodes but rather could be compared with a carcinomatous pneumonia.

*Diagnosis.* Epidermoid bronchial carcinoma.

*Case* 3

A seventeen-year-old male cross-bred dog was examined post-mortem.

*Macroscopic appearance.* The right diaphragmatic lobe was enlarged, though maintaining its shape. It was of firm consistency and of grayish colour. The cut surface showed that the parenchyma was transformed

into a neoplastic mass, presenting two different aspects. The hilar region was of yellowish colour and granular appearance and showed a great number of cavities of varying size and irregular outline. The walls of these cavities were also granular and in some instances passages between one another or leading to the bronchi could be seen. The lower half of the lobe was whitish-gray in colour and solid and homogeneous in appearance. The whole lobe was infiltrated by the tumour tissue, so that no normal parenchyma could be distinguished. The pleural covering remained intact though thickened. There was a rounded grayish-white firm lesion, approximately 1.5 cm. in diameter, in the right cardiac lobe, near its inferior border. It was covered by the pleura, making a slight bulge on the surface. The cut surface was well defined in outline, of whitish colour and granular appearance, and showed a small central cavity. Near the apex of the left diaphragmatic lobe there was a subpleural nodule, about the size of a lentil, of firm consistency and grayishs white colour. The cut surface showed a whitish colour and a homogeneou- appearance and the edge was well defined.

The posterior mediastinal lymph nodes were enlarged. When cut, a whitish nodule was seen, 1 cm. in diameter, located at the posterior end' surrounded by a narrow rim of lymphoid tissue. The heart showed an irregular area— a recent anaemic infarct—near the left part of the coronary sulcus. In the liver there were many metastatic nodules of different sizes distributed through all lobes and small tumour nodules were found throughout the spleen. Both kidneys showed anaemic infarcts; some seemed to be quite recent, whereas in others cicatricial contraction had occurred.

*Histological appearance.* The various sections of the lung showed a tumour of acinar pattern. Some of the acini were solid, others had a central lumen. Most of the bronchi and bronchioli were normal but in a few of them the lining was stratified and the cells had a neoplastic appearance. In one section a proliferation with a pseudopapillary appearance was found in the wall of a bronchiole. The cells of this proliferation as well as those of the alveolar lining and those of the solid acini were large, mostly polyhedral, with a homogeneous and slightly basophilic cytoplasm. Tonofibrils could not be demonstrated between them. The nuclei were mostly rounded, their chromatin varying from very dense to very loose. Some nuclei were oval or triangular in shape, while some were inclined to be spindle-shaped and some distorted nuclei were seen. In some instances pyknosis, karyorrhexis or karyolysis were also present.

Between these cells, there were in certain fields some columnar cells whose nuclei were elongated and placed in the lower third of the cell. From one to four nucleoli were present, the cells with one nucleolus only predominating. The various stages of mitosis were easily found.

Some lung alveoli were lined with columnar cells only. Tumour cells were found at some places lining a lung alveolus (Fig. 4), although its wall remained intact. This indicates that the growth

had not been a destructive one but was merely invading the alveoli. The detached septal cells, although free in the alveolar lumen, could easily be distinguished from the tumour cells, for they were smaller, the cytoplasm was more eosinophilic and their shape was much more regular. The stroma was delicate and mostly represented by the normal pulmonary tissue. Silver methods showed that reticular fibres were present in the stroma but not between the tumour cells.

The histological appearance of the metastases was the same as that of the pulmonary tumour. Tumour masses were found growing in blood vessels in the heart, spleen and kidneys. The parenchyma surrounding these vessels was usually necrotic. In the kidney especially, very many blood vessels contained tumour buds (Fig. 5) and, in addition, neoplastic cells were found inside the glomerular capillaries. Tumour buds growing between the tubules spread towards the medulla. The appearance of an epidermoid carcinoma was seen very typically in the renal metastases. In one field only, the columnar cells appeared to be ciliated.

*Diagnosis.* Bronchial epidermoid carcinoma.

*Case* 4

A male dog of no definite breed, aged about 10 years, was examined post-mortem.

*Macroscopic appearance.* The lungs as a whole had a quite normal appearance with the exception of a few small nodules that were found at the edge of one of the lobes. The nodules were firm, whitish-gray and a little smaller than a lentil. The cut surface of the lungs was pinkish red; the bronchi contained a mucous substance. In the liver necrotic areas, up to 2 cm. in diameter and light brown in colour, were present. Small haematomas were found in the spleen. and the kidneys were congested. No metastatic growths were noticeable.

*Histological appearance.* In most of the sections the lung showed its normal structure. In sections of the nodules, the structure of the alveoli was still present but they were lined by one or more layers of polyhedral cells (Fig. 6) resembling epidermal epithelium. However, there were no tonofibrils between the cells. The bronchioli of this area were lined with the same type of cells; they could be distinguished from the alveoli by their much wider lumen and also by their muscular walls. With high magnification, the cells were seen to be mostly polyhedral but some were rounded or even elongated. Their nuclei were mostly rounded but there were also elongated, triangular and amorphous ones, of different sizes, and giant or irregularly shaped nuclei were rather frequent. Chromatin was mostly loose but some nuclei were pyknotic. Most of the nuclei had one or two nucleoli but more could be seen in some. The different phases of mitosis were abundant. Many neoplastic cells were in different stages of degeneration.

The stroma was delicate and was represented generally by the lung tissue, which was thickened in some fields and infiltrated by leukocytes, especially lymphocytes, and between these there was also a slight infiltration of tumour cells. In many fields the blood vessels were distended and filled with blood. The subpleural connective tissue was thickened and a distinct elastic membrane separated it from the parenchyma. The cells of the pleural serosa were hypertrophied and desquamating. No reticulin was found between the neoplastic cells.

*Diagnosis.* Bronchiolar epidermoid carcinoma.

*Case* 5

A five-year-old male dog of no definite breed was examined post-mortem.

*Macroscopic appearance.* A great number of very small nodules (the size of a pin head or slightly bigger) were scattered throughout the lung tissue of the left diaphragmatic lobe. Those beneath the pleura formed a small bulge on the surface, where they were covered by the moderately thickened serosa. All nodules were of firm consistency and grayish colour. The cut surface showed the same colour and the edges of the growths were well-defined.

No other tumours were present in any other organ.

*Histological appearance.* Microscopic examination revealed numerous rounded foci. They were seen both underneath the pleura and in the central part of the lobe, the parenchyma around them being collapsed. In a few of the nodules only did the cells form cords. In the rest they were packed in masses without definite structure. Some of the cells forming cords were cubical but most of the cells were polyhedral, while some were rounded. The cytoplasm was abundant, homogeneous and slightly basophilic. The nuclei were rounded, or sometimes slightly elongated. No nucleoli were seen inside the nuclei and no cells in mitosis were found. In some of the alveoli two or three cell layers were present, thus forming a papilla in the alveolar lumen. This represents the beginning of a septal proliferation. When stained by the van Gieson method, a delicate connective and vascular tissue of collagenous fibrils was revealed. Silver impregnation revealed the reticular tissue, forming either a net, or isolated fibrils and fibril fragments between the tumour cells (Fig. 7). The tumour foci were well circumscribed.

*Diagnosis.* Histiocytoma.

## DISCUSSION

A study of the cases described above suggested some conclusions concerning the problem of lung cancer and the histogenesis of the alveolar cells which support the opinion of various other authors.

In case 1, the dominating structure encountered is that of a bronchial carcinoma, consisting of columnar cells. Groups of spindle-shaped and of round cells were also present however, indicating a lack of complete differentiation of the tumour. Upon detailed examination, all intermediate cell shapes could be found. This fact suggests that both oat-cell carcinomas, which some authors describe as spindle-cell sarcomas, and undifferentiated round-cell carcinomas, formerly taken to be round cell sarcomas, may be epithelial neoplasms, originating from the bronchi. This opinion is also that of Willis (1948), who found a great number of associated morphological types among his cases of lung carcinoma. The pulmonary neoplasms of columnar cells, as described here, have been looked upon by the majority of pathologists as arising from the bronchial epithelium.

The main structure of cases 2 and 3 was found to be very similar to that of squamous carcinomas, hence the term bronchial epidermoid carcinoma. In case 4 the structure was similar but the growth was connected more to the bronchioli. Those pathologists who have observed this type of malignancy do not agree as to its histogenesis. Thus, some writers including Siegart (1893), Tabusso (cited by Chierici, 1911) and Stazzi (cited by Cella, 1937) believe that it arises from the "alveolar epithelium", which, they say, had previously undergone metaplastic changes. Others think that it originates from the bronchi, the columnar epithelium of which was first transformed into a stratified squamous one. Both possibilities are accepted by some authorities (Aschoff, 1950).

In case 3, columnar cells were seen in some fields, and in the kidney, some cells seemed to be ciliated. Many alveoli were lined with neoplastic cells, without affecting the integrity of their walls. The detached septal cells floating loosely in the alveolar lumen could easily be distinguished from the loose tumour cells. The sections show that the neoplasm invaded the alveoli, where the cells became polyhedral, but that its true origin is really the bronchial epithelium. Thus case 3 suggests that the epidermoid metaplasia may occur in the tumour tissue itself, as had been already realised by Kretschmar (cited by Ewing, 1948) and Ewing (1948).

The morphology of case 5 is identical with that found by many authors, including Grady and Stewart (1940) and Mostofi and Larsen (1951), in experimentally induced lung tumours of mice. These authors were able to prove that these tumours arose from the alveolar lining but they do not establish the nature of these cells. In the present case, reticulin was revealed between the tumour cells by silver impregnation methods. This is strongly suggestive of the fact that this tumour has a mesenchymal origin. Bonnamour (cited by Huguenin, 1928) expressed the same opinion when he described a tumour of small cells.

The problem concerning the nature of neoplasms arising from the alveolar cells is a very complex one, and it is closely linked with that of the origin of these cells. There is no agreement among histologists regarding this point. While Chiarugi (1940), Lordy, Oria and Aquino (1940) and others believe that they are endodermic, Fried (cited by Rose, 1928), Rose (1928), Policard and Muller (1944) and Policard (1947, 1950) tried in a series of studies to prove their mesodermal nature.

Pathologists also do not agree as to the true nature of the alveolar lining, which is said by different authors to give rise to the various tumours of the lung.

Of the cases described here, two produced metastases—in case 1 in the brain only, in case 3 in various organs. The metastases were apparently blood-borne—in the first case because no lymphatics exist in the C.N.S., in the second, because numerous blood vessels were found obstructed by neoplastic cells.

In the opinion of almost all human pathologists, the frequency of primary carcinomas of the lung has been rapidly increasing in recent years. In the veterinary field Cohrs (Nieberle and Cohrs, 1949) also accepts an increase in frequency, whereas most other authors believe that primary pulmonary malignancies are very rare in animals. We not propose to discuss this matter at the moment but to leave the analysis of this problem for another paper which is being prepared, based on statistical data from our São Paulo archives.

## CONCLUSIONS

Five primary lung tumours of dogs are reported, their gross and microscopic descriptions being given.

The first case is of a bronchial columnar-cell carcinoma showing undifferentiated areas resembling spindle-cell and round-cell sarcomas.

There are three cases of epidermoid bronchial carcinomas. In one of these, evidence was found that the squamous metaplastic change had occurred in the tumour tissue itself.

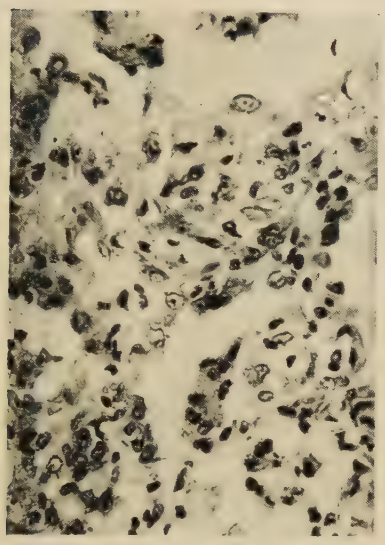
The fifth case is classified as a histiocytoma from its morphology as well as from the presence of reticulin between the cells. It probably originated from the alveolar cells.

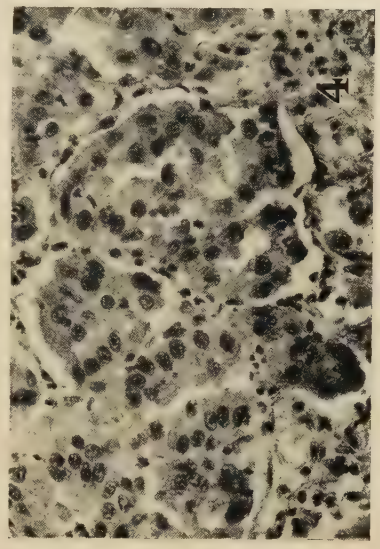
## REFERENCES

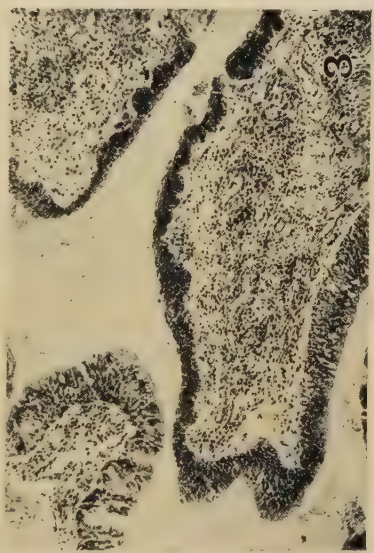
Aschoff, L. (1950). *Tratado de Anatomia Patologica.*, Vol. 2, p. 326. 2nd ed. Labor, S.A. Barcelona.

Cella, F. (1937). *Nuovo Vet.*, **15**, 196.

Chiarugi, G. (1940). *Trattato di embriologia.* p. 297. Soc. Ed. Libraria, Milano.





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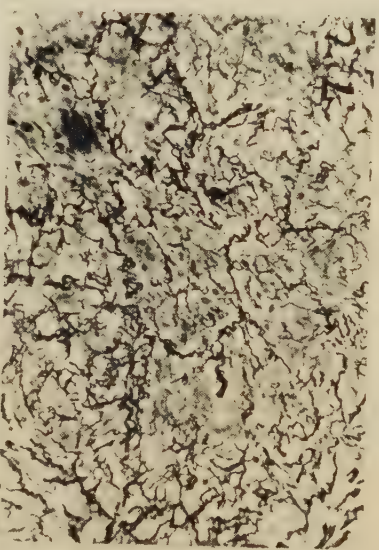

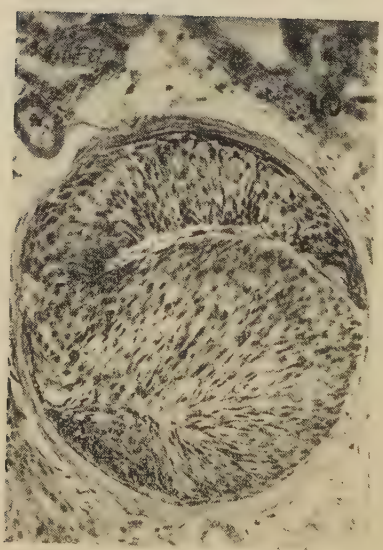

Chierici, G. (1911). *Nuovo Erc.*, **86**, 103.
Drieux, H., and Thiery, G. (1945). *Bull. Acad. vet. Fr.*, **18**, 276.
Ewing, J. (1948). *Oncologia.* p. 918 Barcelona, Salvet, Ed. S.A.
Giannini, G. (1948). *Riv. Anat. Pat. e Oncol.*, **1**, 1.
Grady, H. G., and Stewart, H. L. (1940). *Amer. J. Path.*, **16**, 417.
Huguenin, R. (1928). *Le cancer primitif du poumon.* Masson et Cie, Paris.
Jackson, C. (1936). *Onderstepoort J. vet. Sci.*, **6**, 126.
Lordy, C., Oria, J., and Aquino, J. T. (1940). *Embriologia humana e comparada.* p. 410 Cia. Melhoramentos, São Paulo.
Monlux, W. S. (1952). *Primary pulmonary neoplasms in domestic animals. The Southwestern Veterinarian.* October, 1952.
Mostofi, F. K., and Larsen, C. D. (1951). *J. nat. Cancer Inst.*, **11**, 1187.
Nieberle, K. and Cohrs, P. (1949). *Lehrbuch der speziellen pathologischen Anatomie der Haustiere.* p. 170 3rd ed. Gustav Fischer, Jena.
Policard, A., and Muller, F. (1944). *Bull. Histol. Appl.*, **21**, 67.
Policard, A. (1947). *Ibid.*, **24**, 127; (1950) *Ibid.*, **27**, 47.
Rievel, — (1906). *Dtsch. tierärztl. Wschr.*, **14**, 121.
Rose, S. B. (1928). *Arch. Path.*, **6**, 36.
Schlotthauer, C. F., and Millar, J. A. S. (1951). *J. Amer. vet. med. Ass.*, **119**, 442.
Siegert, F. (1893), *Virchows Arch.*, **134**, 287.
Willis, R. A. (1948). *Pathology of tumours.* p. 359 Butterworth and Co., London.

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## KEY TO FIGURES

Fig. 1. Case 1. Neoplastic growth imitating bronchioli. Bielschowsky × 1,000
Fig. 2. Case 2. Neoplastic cells lining pulmonary alveoli and infiltrating stroma. H & E × 1,000
Fig. 3. Case 2. Bronchus showing squamous metaplasia and infiltration of neoplastic cells. H & E × 250
Fig. 4. Case 3. Neoplastic cells invading pulmonary alveoli. H & E × 1,000
Fig. 5. Case 3. Renal metastasis of bronchial epidermoid carcinoma obstructing a blood vessel. H & E × 1,000
Fig. 6. Case 4. Alveoli lined by neoplastic cells. H & E × 250
Fig. 7. Case 5. Neoplastic nodule showing reticulin Bielschowsky × 250

# IMMUNISATION AGAINST CONTAGIOUS BOVINE PLEUROPNEUMONIA, WITH SPECIAL REFERENCE TO THE USE OF A DRIED VACCINE

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## INTRODUCTION

To workers on pleuropneumonia, particularly those in tropical and undeveloped countries, the disadvantages of culture vaccines are too well known to need emphasis. There is a continuous fall in numbers of viable organisms and, therefore, of immunising power as the vaccine ages; the effective "life" of such vaccines is, at most, three weeks, and no two batches, almost indeed no two cultures, can be regarded as identical in attenuation.

It has, thus, been clear for many years that some form of standard product was desirable. In a country like the Sudan, a standard product is required which can be kept for at least a year in the ice-chest followed by at least a month at 37°C. The ultimate object of the work recorded in this paper was the production of a dried vaccine which would satisfactorily immunise cattle against contagious bovine pleuropneumonia.

## RESULTS

### *Initial Experiments on Tail Inoculation*

In an attempt to produce a more powerful immunity than that provided by Bennett's method of vaccination, recourse was had to the tail-tip method of inoculation so widely practised in Australia (Seddon, 1953).

Pairs of young bulls were inoculated with 0.2 ml. of a 2-day serum broth culture of organisms subcultured every 2nd day for a varying number of times. The results with one strain are shown in Table 1.

All animals in this and subsequent experiments were negative to the flocculation test (Priestley, 1951). They were re-tested 14 days after vaccination and those which were still negative again on the 21st day. Four weeks after vaccination the animals were challenged by the injection of 1ml. of virulent culture intramuscularly at the shoulder. It will be seen that with unstored vaccine, the 5th (bulls 1, 2), the 10th (bulls 23, 24) and the 15th (bulls 31, 32) subcultures immunised while the 20th (bulls 39, 40) did not. Another strain tested in much the same way immunised cattle with subcultures up to the 20th but not with the 30th.

TABLE 1

IMMUNITY FOLLOWING INOCULATION INTO THE TAIL OF VACCINE STORED FOR VARIOUS TIMES

| *Subculture tested* | *Days stored in* | | *Bull No.* | *Results of* | |
|---|---|---|---|---|---|
| | *ice-chest* | *incubator* | | *Flocculation* | *Challenge* |
| 5th | 0 | 0 | 1 | + | i |
| | | | 2 | + | i |
| | | 14 | 3 | + | i |
| | | | 4 | + | i |
| | | 21 | 5 | — | nsd |
| | | | 6 | — | nsd |
| | 14 | 0 | 7 | + | i |
| | | | 8 | — | nsd |
| | | 7 | 9 | — | rd24 |
| | | | 10 | — | rs |
| | | 14 | 11 | — | rs |
| | | | 12 | — | nsd |
| | | 21 | 13 | — | i |
| | | | 14 | — | i |
| | 28 | 0 | 15 | — | rd14 |
| | | | 16 | — | rd20 |
| | | 7 | 17 | — | rk |
| | | | 18 | — | i |
| | | 14 | 19 | — | rs |
| | | | 20 | — | nsd |
| | | 21 | 21 | — | i |
| | | | 22 | — | i |
| 10th | 0 | 0 | 23 | + | i |
| | | | 24 | + | i |
| | | 7 | 25 | — | i |
| | | | 26 | — | rk |
| | | 14 | 27 | + | i |
| | | | 28 | — | rd30 |
| | | 21 | 29 | — | rd33 |
| | | | 30 | — | rs |
| 15th | 0 | 0 | 31 | + | i |
| | | | 32 | — | i |
| | | 7 | 33 | — | rd24 |
| | | | 34 | — | rs |
| | | 14 | 35 | — | rs |
| | | | 36 | 0 | nsd |
| | | 21 | 37 | + | i |
| | | | 38 | 0 | i |

TABLE 1 (*Cont.*)

| *Subculture tested* | *Days stored in* | | *Bull No.* | *Results of* | |
|---|---|---|---|---|---|
| | *ice-chest* | *incubator* | | *Flocculation* | *Challenge* |
| 20th | 0 | 0 | 39 | + | rd28 |
| | | | 40 | — | nsd |
| | | 7 | 41 | — | i |
| | | | 42 | — | i |
| | | 14 | 43 | + | i |
| | | | 44 | — | rs |
| | | 21 | 45 | — | rs |
| | | | 46 | — | i |

+ = flocculation positive; — = flocculation negative; i = immune to challenge; rd24 = reacted and died 24 days after challenge; rs = reacted but survived; rk = reacted and killed moribund; nsd = died from other causes before test. 0 = not tested.

There were few severe tail reactions and the results appeared to be encouraging. However, as might have been expected, it was soon shown that the "life" of such a vaccine was no better than that of more attenuated ones inoculated subcutaneously in a larger dose. Thus, as may also be seen from Table 1, a 5th subculture immunised after storage for 14 days in the incubator (bulls 3, 4), but a 10th subculture failed to immunise even after only 7 days storage (bulls 25, 26). Similarly a 5th subculture stored for 14 days in the ice-chest immunised one of the two bulls (7, 8) inoculated immediately but failed to immunise if it was stored for a further period of 7 days in the incubator (bulls 9, 10).

A further test of this method on a larger scale and using a different strain was carried out in the southern Sudan (Malakal). One hundred young bulls were inoculated in the tail with 0.2 ml. of a 5th subculture vaccine which, after preparation in the usual way, had been stored at incubator temperature for 13 days, (i.e. the total time of incubation had been 15 days.)

Four animals died from liver fluke infection very soon after vaccination. The remaining 96 were bled and flocculation tested 19 days after vaccination; 68 gave positive and 14 doubtful reactions while 14 were negative. Twenty-one of these animals developed tail reactions of varying degrees of severity and two of them died before challenge; 5 more died of other causes before challenge. The surviving 89 vaccinated animals and 8 unvaccinated controls were challenged 27 days after vaccination. The results are summarised in Table 2 from which it may be seen that whereas 4 of the controls were positive only 6 of the vaccinated animals reacted.

The difference is statistically significant, the value for $p$ in the $x^2$ test being very much less than 0.1.

TABLE 2

FURTHER OBSERVATIONS ON IMMUNISATION WITH STORED VACCINE

| *Class of animal* | *Results of challenge* | |
|---|---|---|
| | *reacted* | *immune* |
| Flocc. positive .. | 1 | 63 |
| ,, doubtful .. | 3 | 10 |
| ,, negative .. | 2 | 10 |
| Controls .. .. | 4 | 4 |

Thus while the tail-tip method appeared to be an advance in that vaccine production would be simpler and quicker and immunisation probably more efficient, there was still a problem of the limited "life" of the vaccine.

*Experiments on Vaccination with Dried Organisms*

In view of the results recorded above the possible use of dried organisms was subjected to further study. It has been known for some time that the pleuropneumonia organism can be maintained viable in the dried state for many years (Priestley, Mihimeid and Dafaalla, 1948) but such dried organisms fail to establish themselves when inoculated into cattle and thus fail to set up immunity (Priestley, 1952). It seemed clear that if dried organisms were to survive in the tissues they would have to be inoculated with an adjuvant or protective substance.

Of the few substances used in preliminary trials the one which gave the most promising results was powdered agar (Difco-bacto) and further studies using this substance were therefore carried out.

*Dried organisms with adjuvant.* Briefly, a vaccine was prepared by reconstituting the dried organisms in a suitable fluid and immediately mixing with 1 per cent agar melted and cooled to below 50°C. After the agar had set, animals were inoculated in the tail with 0.2 ml. For this experiment dried organisms from a 3rd subculture were used. Eighteen bulls were vaccinated. Twelve gave positive flocculation reactions 14 to 21 days later : of these, one developed a severe tail reaction, which spread to the buttocks and then forward along the ribs, from which it died 31 days later. Another of the flocculation positive and two of the flocculation negative animals died from causes other than pleuropneumonia before challenge. The surviving 14 animals and 2 controls were

challenged 23 days after vaccination. All 10 flocculation positive and 2 of the flocculation negative animals were immune. The remaining two flocculation negative animals reacted severely and died 30 and 32 days later respectively. Of the 2 controls, one was killed *in extremis* 21 days after inoculation and the other died the next day. The results of the challenge 23 days after vaccination, are summarised in Table 3. It should be emphasised that not only was there very definite evidence of immunity but that the immunity was established very much more quickly than is the case when highly attenuated cultures are inoculated subcutaneously.

TABLE 3

IMMUNISATION WITH DRIED ORGANISMS SUSPENDED IN 0.5% AGAR

| *Class of animal* | *Results of challenge* | |
|---|---|---|
| | *Nos. immune* | *Nos. reacted* |
| Flocc. positive .. | 10 | 0 |
| ,, negative .. | 2 | 2 |
| Controls .. | .. 0 | 2 |

*Comparison of aqueous and adjuvant vaccines.* At this point it was, perhaps, not clear whether the survival of the dried organisms was due to the adjuvant or to the fact that they were more virulent (i.e. had been subcultured fewer times) than had been the case in the past. However the next experiment gave an unequivocal answer.

Dried organisms from the 5th, 10th, 16th and 20th subcultures respectively were resuspended in the usual way and the suspensions divided into two lots. To one was added an equal volume of water and to the other an equal volume of melted and cooled 1 per cent agar. Groups of 5 bulls were inoculated with the eight vaccines. Flocculation tests were carried out 14 and 21 days after vaccination and the animals were challenged with virulent culture 23 days after vaccination. The results of the experiment are shown, in detail, in Table 4.

The difference in the effects of the two sets of vaccines was very striking. Only one animal (71) inoculated with watery vaccine developed any flocculating antibody before challenge, though another (67) appeared to have reacted to the vaccine since it developed a severe tail reaction which did not start until after the inoculation of the challenge dose; one animal (73) appeared to be non-specifically resistant. On the other hand all animals inoculated with 5th, 10th and 16th subcultures in agar developed marked flocculation reactions and showed no reaction to the challenge dose. Of the five animals inoculated with the 20th subculture in agar, one

TABLE 4

THE PROTECTIVE EFFECT OF AGAR ON INOCULATED DRIED ORGANISMS

| Sub-culture tested | Dried orgs. in water | | | | Dried orgs. in 0.5% agar | | | |
|---|---|---|---|---|---|---|---|---|
| | Bull No. | Flocc. test days after vacc. | | Challenge result | Bull No. | Flocc. test days after vacc. | | Challenge result |
| | | 14 | 21 | | | 14 | 21 | |
| 20th | 61 | — | — | rd20 | 81 | o | o | nsd |
| | 62 | — | — | rd26 | 82 | — | — | rs |
| | 63 | — | — | rd24 | 83 | ++ | o | i |
| | 64 | — | — | rd26 | 84 | — | + | i |
| | 65 | — | — | rd26 | 85 | + | +++ | rd27 |
| 16th | 66 | — | — | rd26 | 86 | ++ | o | i |
| | 67 | — | — | kt | 87 | +++ | o | i |
| | 68 | — | — | rd29 | 88 | — | +++ | i |
| | 69 | — | — | rs | 89 | — | + | i |
| | 70 | — | — | rs | 90 | — | ++ | i |
| 10th | 71 | — | ++ | i | 91 | +++ | o | i |
| | 72 | — | — | rs | 92 | +++ | o | i |
| | 73 | — | — | i | 93 | +++ | o | i |
| | 74 | — | — | rd35 | 94 | +++ | o | i |
| | 75 | — | — | rd35 | 95 | +++ | o | i |
| 5th | 76 | — | — | rd27 | 96 | +++ | o | i |
| | 77 | — | — | rd38 | 97 | +++ | o | i |
| | 78 | — | — | rs | 98 | +++ | o | i |
| | 79 | — | — | rs | 99 | +++ | o | i |
| | 80 | — | — | rd22 | 100 | +++ | o | i |

kt = killed with a severe tail reaction.
For remaining abbreviations see Table 1.

died of Theileria infection a few days after the experiment started, two developed antibody and were immune, one developed no antibody and reacted and, surprisingly, one (85) developed antibody yet died 27 days after challenge.

It may be worth noting here that the strain used for this experiment was the same as that used for the experiment summarised in Table 1. It will be seen that with the dried vaccine highly satisfactory immunity was obtained with the 16th subculture while the 20th was not so good; with the "wet" vaccine immunity was obtained with a 15th subculture but not with a 20th. If this comparison is a true representation and can be confirmed it would mean that it would be a comparatively simple matter to determine beforehand the subculture suitable for the preparation of dried vaccine.

*Immunisation with cultures stored one month.* With the object of confirming and extending the above findings further experiments were carried out on cattle in the southern Sudan.

A group of 140 flocculation-negative cattle were purchased. Ten of these were inoculated in the tail with dried 16th sub-culture organisms which had been stored for one month in the ice-chest before being reconstituted in agar; a further ten were inoculated with similar vaccine, the dried organisms of which had been stored for one month in the incubator; and ten were kept as controls. Of the remainder, 55 were inoculated with dried 10th subculture stored for one month in the ice-chest and 55 with 10th subculture stored in the incubator, both vaccines being reconstituted in agar. There was some delay in carrying out these inoculations and they were not finished until about three hours after the vaccine had been reconstituted.

Fourteen days later the 130 vaccinated animals were bled and tested for flocculating antibody. Only 53 reacted and most of the reactions were only weak or doubtful ones. One month after vaccination all 20 animals inoculated with 16th subcultures, 20 animals inoculated with 10th subcultures and the 10 controls were challenged with virulent culture. The results (Table 5) were disappointing and it was not until further work (see page 176) had been done that the explanation was forthcoming.) It will be seen that of the 39 vaccinated animals (one died from other causes) no fewer than 26 reacted and of these 16 died; at the same time 9 out of the 10 controls reacted and of these 6 died. The difference in survival rates is not statistically significant.

TABLE 5

IMMUNISATION WITH DRIED ORGANISMS STORED FOR ONE MONTH

| *Vaccine* | *Results of challenge* | |
|---|---|---|
| | *No. reacting* | *No. immune* |
| 16th sub-stored in ice-chest .. | 6 | 4 |
| „ „ incubator .. | 7 | 3 |
| 10th „ ice-chest .. | 7 | 3 |
| „ „ incubator .. | 6 | 3 |
| Controls .. .. .. | 9 | 1 |

*Immunisation with cultures stored two months.* After storage in the incubator and in the ice-chest for two months, organisms from the 5th subculture were reconstituted in agar and injected into the tails of groups of three cattle. Some were injected within 15 minutes of the preparation of the vaccine and some after it had been standing for two hours. Seven, 14 and 21 days later some or all of the animals were bled and tested for flocculating antibody with the results shown in Table 6.

It is thus clear that the dried organisms survived for over two months even when stored at incubator temperature and that organisms reconstituted in agar in saline survived for over two hours (see page 177).

TABLE 6

ANTIBODY PRODUCTION FOLLOWING INOCULATION OF DRIED ORGANISMS STORED FOR TWO MONTHS

| *Vaccine stored in* | *Time of injection after preparation* | *Bull No.* | *Flocculation results after (days)* | | |
|---|---|---|---|---|---|
| | | | 7 | 14 | 21 |
| ice-chest | 15 mins. | 101 | + | +++ | 0 |
| | | 102 | — | +++ | 0 |
| | | 103 | — | +++ | 0 |
| | 2 hours | 104 | +++ | +++ | 0 |
| | | 105 | + | +++ | 0 |
| | | 106 | 0 | 0 | 0 |
| incubator | 15 mins. | 107 | — | +++ | 0 |
| | | 108 | — | +++ | 0 |
| | | 109 | — | ± | +++ |
| | 2 hours | 110 | + | ++± | 0 |
| | | 111 | — | +++ | 0 |
| | | 112 | — | ± | +++ |

It was not regarded as necessary to challenge these animals and they are being kept for further experiments on the duration of immunity.

*Reactions following tail vaccination.* A number of animals reacted more or less severely to the inoculation of the dried, agar reconstituted vaccine, but there appeared to be little or no relationship between virulence and reaction. Rather the reactions seemed to be related to individual suspectibility. Thus of the animals inoculated with dried 3rd subculture organisms, two reacted severely and one of these died; four more gave mild reactions and recovered. Of the animals referred to in the experiment on the comparison of aqueous or agar vaccines one inoculated with 16th subculture in agar, one inoculated with 20th subculture in agar and one inoculated with 16th subculture in water reacted severely; all the animals inoculated with 5th and 10th subcultures in agar, on the other hand, reacted mildly and transiently at the tip of the tail but recovered rapidly.

One of the flocculation postive, immune animals inoculated with dried 3rd subculture organisms in agar was found, when killed for other purposes, to contain a very small abscess in its lungs and from this pleuropneumonia organisms were cultured. Whether this lesion resulted from the inoculation or not it is now impossible to say, but in view of this finding, and bearing in mind the work of Daubney (1935), Mettam and Ford (1939) and others, the possibility of lung infarcts following tail injection of dried vaccine in agar was borne in mind. The majority of the locally available experimental animals have since been killed and carefully examined and no further lung lesions have been seen.

*The Experiments on the Survival of Organisms during the Process of Vaccine Production*

In experiments so far described the dried organisms were reconstituted in water and mixed with agar dissolved in water. In view of the poor flocculation results given by the animals listed in Table 5, experiments were carried out to determine the survival rates at various stages in the process of vaccine production and use.

*Survival of undried organisms in distilled water.* Approximately 100 ml. of a 2-day serum broth culture were centrifuged at 2,500 r.p.m. for 2 hours. The supernatant fluid was discarded and the deposited organisms resuspended in 10 ml. of sterile distilled water. Using 1 ml. amounts of the suspension, dilution counts were made at intervals. There appeared to be little drop in count from an initial end-point of 1 in $10^{10}$, during the first two hours but by the 24th hour the count had dropped to 1 in $10^{3}$. This result was satisfactory and there appeared to be no need to alter the fluid used for washing off the organisms from the Sharples centrifuge on which they were collected prior to drying. Any fluid other than water might have introduced complicating factors in the drying process.

*Survival in frozen serum.* To reduce variable factors to a minimum it would obviously be desirable to mix before drying as many batches of centrifuged organisms as possible. It was therefore necessary to find a medium in which the organisms would remain viable long as possible. About 40 ml. of a 2-day culture (5th subculture) was centrifuged and the deposited organisms resuspended in 15 ml. of distilled water. To 7 ml. of this suspension was now added another 7 ml. of distilled water, while to a second 7 ml. was added 7 ml. of inactivated horse serum. Amounts of about 1 ml. of the two mixtures were placed in tubes in the deep-freeze cabinet (—30°C). At intervals tubes were thawed in a 37°C water bath and counts made. The results of this experiment are shown in Table 7.

Table 7

Survival of Frozen, Undried Organisms

| *Days suspension stored in deep-freeze* | *Highest dilution showing growth in* | |
|---|---|---|
| | *Water* | 50% serum |
| 0 | $1:10^{8}$ | $1:10^{9}$ |
| 1 | $1:10^{3}$ | $1:10^{9}$ |
| 2 | $1:10^{3}$ | $1:10^{9}$ |
| 5 | 1:10 | $1:10^{7}$* |
| 10 | 1:10 | $1:10^{6}$ |
| 20 | nil | 0 |
| 25 | 0 | $1:10^{6}$ |

*$1:10^{8}$ and $10^{9}$ dilutions contaminated.

It will be seen that there was a sharp drop in viability in distilled water, but in 50 per cent serum the count remained unchanged for at least 48 hours and possibly longer and that even after 25 days there were appreciable numbers of organisms still viable. It would seem therefore that under these conditions it would be quite satisfactory to mix batches of organisms that had not been stored for more than 48 hours.

*The drying technique.* There was never any doubt about the drying procedure. It has previously been recorded that dried organisms will survive for at least 475 days even when stored at 37°C. It may be noted here that quite recently satisfactory growths have been obtained from tubes of dried organisms stored in the ice-chest for 1,629 days ($4\frac{1}{2}$ years) followed by 6 weeks at 37°C.

*Survival of reconstituted dried organisms.* As remarked above it had, at one time, been the custom to reconstitute the dried organisms in water before mixing them with the agar. Experiments on viability showed this to be highly unsatisfactory in that the organisms were killed off very rapidly. Thus in one experiment the count dropped in a matter of minutes from 1 in $10^6$ to 1 in $10^2$ and after half-an-hour no growth was obtained at all. Similarly dried organisms reconstituted in water and then mixed with watery solutions of melted agar were quickly killed off though they did apparently survive a little longer. Thus, in one experiment of this nature, growth was obtained after a few minutes but not after one hour, while in a second experiment growth was obtained after one but not after two hours.

It is probable that reconstitution in 50 per cent serum would be satisfactory but such a procedure would present very considerable practical difficulties under field conditions. It seemed that the two substances which might be used under field conditions would be saline (0.85 per cent) and citrated blood. Accordingly tubes of dried organisms were reconstituted in these and counts done at intervals with the results shown in Table 8 from which it seemed, very fortunately, that saline would be satisfactory.

TABLE 8

SURVIVAL OF DRIED ORGANISMS RECONSTITUTED IN SALINE AND CITRATED BLOOD

| *Suspension counted after (hours)* | *Highest dilution showing growth in* | |
|---|---|---|
| | *Saline* | *Blood* |
| 0 | $1:10^5$ | $1:10^6$ |
| $\frac{1}{2}$ | $1:10^5$ | $1:10^3$ |
| 1 | $1:10^5$ | $1:10^3$ |
| 2 | $1:10^5$ | $1:10^3$ |
| 4 | $1:10^3$ | $1:10^2$ |

As a final check on viability, dried organisms were reconstituted in saline as above and immediately mixed with melted and cooled 1 per cent agar in saline. Counts were done at intervals with the results shown in Table 9. It was at this stage and using the saline technique that the experiment described on page 174 was carried out.

TABLE 9

SURVIVAL OF DRIED ORGANISMS RECONSTITUTED IN AGAR IN SALINE

| *Reconstituting fluid* | *Highest dilution showing growth after (hours)* | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 4 | 8 | 24 |
| Agar in saline .. | $1:10^5$ | $1:10^5$ | $1:10^4$ | $1:10^3$ | nil | nil |

*Preparation of Vaccine*

The method finally adopted for vaccine preparation was as follows.

Tumour "Lymph" from an artificial case of pleuropneumonia is collected under aseptic conditions in small tubes and stored in the deep freeze cabinet at —30°C. It will keep thus for several months and possibly a year or more. When required a tube of the lymph is thawed by immersion in a 37°C water bath and 1 ml. sown into 9 ml. serum broth. From this culture serial 1 in 10 dilutions are made to further 9 ml. volumes of serum broth. Under these conditions there is little or no trouble with contamination but if necessary the majority of the contaminants can be inhibited by adding 0.2 ml. of a solution of 200,000 i.u. of penicillin in 20 ml. of plain broth to each tube of serum broth to give a final concentration of 200 i.u./ml. (White, 1952, 1954).

As soon as microscopically confirmed growth appears in any selected tube in the series, loopful subcultures are made in serum broth and the subculturing is continued in series every 48 hours until that subculture penultimate to the one required is reached. At this point several 1 litre containers of serum broth with penicillin are seeded with a few ml. of the 2-day old subculture. The flasks are incubated for two days and the culture then put through a Sharples centrifuge. The organisms are collected in a small volume of distilled water. Without wasting any time an estimation of the density is made and the suspension standardised, by the addition of more distilled water, to what would be tube 15 of the Brown Scale. Immediately this is done an equal volume of horse serum inactivated at 56°C for ½ hour is added.

If drying is not to be carried out the same day the suspension is placed in the deep freeze for not more than 48 hours. Volumes of 0.1 ml. suspension are placed in the drying tubes and drying is carried out over the 10 day period exactly as described elsewhere (Priestley, 1952). Until used or issued the sealed tubes should be stored in an ice-chest. Under laboratory conditions, before use each tube can be tested for vacuum with a high frequency discharger.

When required, the dried material from one tube is resuspended in 4 ml. of saline. This is immediately added to 4 ml. 1 per cent agar in saline which has been melted and cooled to below 50°C. After setting, which again under laboratory conditions can be hastened by placing in the ice-chest for a few minutes, the vaccine is used as a dose of 0.2 ml. inoculated into the tail about 1 inch from the tip. Vaccine should be used as soon as possible after preparation and any not used within two hours should be discarded.

*The Relationship between a Positive Flocculation Test and Immunity*

It is perhaps desirable to justify the belief that after vaccination a positive flocculation test indicates immunity. No claim is made that the development of flocculating antibody indicates that an animal will be protected against pleuropneumonia. Indeed there is considerable evidence to the contrary. The evidence is not discussed here; suffice it to say that animals dying of pleuropneumonia show considerable quantities of this antibody in their blood. Nevertheless, a positive flocculation test after vaccination must indicate survival and multiplication of the inoculated organisms (Tables 1 and 4). It is also virtually certain that if the organisms can multiply they will set up immunity—provided, of course, that the exposure to infection is not too soon or too severe. Reference to Table 10 will clarify this point. All the animals referred to were inoculated in the tail and were tested for flocculating antibody 2 to 3 weeks later. It will be seen that of the 116 flocculation positive animals 113 were immune on challenge.

TABLE 10

THE RELATIONSHIP BETWEEN FLOCCULATING ANTIBODY AND IMMUNITY

| | *Results of challenge* | | *Totals* |
|---|---|---|---|
| | *Nos. reacted* | *Nos. immune* | |
| Flocc. positive .. | 3 | 113 | 116 |
| ,, negative .. | 48 | 45 | 93 |
| | 51 | 158 | 209 |

## DISCUSSION

The first serious attempt to combat contagious bovine pleuropneumonia in the Sudan was made by Bennett (1932) who used, as vaccine, cultures attenuated by repeated subculture. For 20 years little more than technical improvements were introduced and although production and use of this vaccine have steadily increased from a few thousand doses in 1932 to over 1,500,000 this year, there is little evidence of a decreasing incidence of the disease. In part this is no doubt due to the success of the campaigns against rinderpest and trypanosomiasis; animals which previously died of these diseases now survive to die of pleuropneumonia. There is no

doubt, however, that the vaccine itself is largely at fault since, for instance, many reports come in of cattle dying within a few months of being vaccinated.

At one time it was considered a possibility that as a result of vaccination 12 months previously animals, which were not able to withstand exposure to natural infection, might still have enough residual immunity left to kill off the attenuated organisms inoculated on re-vaccination. In such animals re-vaccination too soon after a primary vaccination might lead to no increase in immunity and thus bring the vaccine into disrepute. Direct experiment (Priestley and Karib, 1954), however, did not support this view for it was shown quite definitely that a second vaccination boosted the existing immunity.

A new approach to the problem was recently made by Sheriff and Piercy (1952, 1953) who claimed to have specifically attenuated a particular strain of the pleuropneumonia organism by passaging it through fertile eggs and to have successfully immunised cattle with "avianised" vaccine. Dafaalla and White (1953) criticised this work on theoretical grounds and this year Dafaalla and Nasri (1954) have been unable to confirm the findings. They were able to adapt a virulent strain to fertile eggs by yolk to yolk passage but after 45 passages there was little evidence of attenuation. Furthermore, under Sudan conditions, the duration of immunity, following inoculation of dried avianised vaccine received from Kabete, was too short to be of practical value. Thus in Barh El Ghazal Province, where 1,220 cattle were vaccinated, 206 (16.9 per cent) showed clinical evidence of pleuropneumonia within 5 to 6 months and 195 deaths were reported. Similarly in Kordofan Province, where 474 cattle were vaccinated, 18 typical cases of pleuropneumonia were seen within a few months and the owners were even asking for re-vaccination with culture vaccine. It is possible, though the writer thinks it unlikely, that improved methods of production may lead to an improved vaccine (see Priestley, 1954).

Thus of the various methods of immunisation available the most satisfactory appears to be that of inoculating relatively virulent organisms into the tail. The method has, at any rate, been used with considerable success in Australia. Immunity is set up very quickly. Campbell (1938), for instance, believes that animals may be immune 3 to 4 days after vaccination and, judging by our experience, they may certainly be immunised within 23 and probably within 14 days of vaccination. Immunity also lasts for a considerable time; thus it has been shown that animals vaccinated three years previously were immune (Seddon, 1953). As has been noted, the disadvantage of the method, as it stands, is the short effective "life" of the vaccine. By using dried organisms suitably reconstituted it is now believed that this objection has been overcome.

With the onset of the cool, dry weather it is hoped to produce sufficient dried vaccine to give the method an extended field trial.

During the course of this work Gray and Turner (1954) reported successful results with a dried vaccine without an adjuvant. In view of the repeatedly confirmed results reported here and elsewhere that dried organisms do not survive in the tissues unless an adjuvant is added, their results are surprising. Their technique, however, was substantially different since they dried serum broth cultures direct and herein may lie the explanation of the differences reported in the present article. For large scale production, the technique of Gray and Turner would, in any case, be impractical. To produce, say, two million doses by their method would necessitate the drying of a bulk of 400 litres, whereas by the present method of concentrating the organisms, the bulk can be reduced to 5 litres: it is probable that it can be reduced still further.

The adjuvant used in these experiments is by no means ideal. The preparation of the vaccine will present difficulties under field conditions and, clearly, reconstitution cannot be left in the hands of illiterate and untrained stockmen. The value of the work reported here and of that of Gray and Turner lies in the finding that a dried vaccine can be used. No doubt further work will reveal a more suitable adjuvant.

## CONCLUSIONS

Confirmatory observations have been made on the immunisation of cattle against contagious bovine pleuropneumonia using the Australian method of inoculating relatively virulent cultures into the tail.

It has been confirmed that dried organisms reconstituted in water, broth and various other fluids do not immunise, presumably because they are unable to establish themselves in the tissues. On the other hand, dried organisms reconstituted in agar solutions immunise efficiently and very rapidly.

There is direct evidence that cattle can be immunised with vaccine stored in the dried state for two months and indirect evidence that the vaccine will be satisfactory for at least $4\frac{1}{2}$ years.

Reconstituted vaccine does not survive for very long and should be used within two hours.

## ACKNOWLEDGMENTS

It was largely as a result of the stimulating criticism and suggestions made to us by Dr. T. S. Gregory, Animal Health Laboratory, C.S.I.R.O., Australia who visited Khartoum late in 1953, that work on the tail method of vaccination was started; and it was this which led on to work with agar.

I am greatly indebted to my Director, Mr. J. T. R. Evans, who has continually encouraged and advised me. This paper is published with his approval.

## REFERENCES

Bennett, S. C. J. (1932). *J. comp. Path.*, **45**, 257.
Campbell, A. D. (1938). *J. Council sci. industr. Res. Aust.*, **10**, 164.
Dafaalla, N., and White, R. W. (1953). *Vet. Rec.*, **65**, 147.
Dafaalla N., and Nasri. (1954). *Dept. Anim. Production, Khartoum.* (in press).
Daubney, R. (1935). *J. comp. Path.*, **48**, 83.
Gray, D. F., and Turner, A. W. (1954). *Ibid.*, **64**, 116.
Mettam, R. W., and Ford, J. (1939). *Ibid.*, **52**, 15.
Priestley, F. W. (1951). *Vet. Rec.*, **63**, 427; (1952). *J. comp. Path.*, **62**, 125; (1954). *Brit. vet. J.* (in press).
Priestley, F. W., and Karib, A. A. (1954). *Vet. Rec.*, **66**, 310.
Priestley, F. W., Mihimeid, M. A., and Dafaalla, N. (1948). *Ibid.*, **60**, 203.
Seddon, H. R. (1953) *Commonwealth of Australia, Service Publ.* (*Div. Vet. Hyg.*) No. 10, *Diseases of Domestic Animals in Australia*, Pt. 5, ii., 360.
Sheriff, D., and Piercy, S. E. (1952). *Vet. Rec.*, **64**, 615; (1953) *Proc. XVth Int. Vet. Congr. i*, 333.
White, R. W. (1952). *Brit. vet. J.*, **108**, 111; (1954) *Vet. Rec.*, **66**, 293.

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# *PASTEURELLA HAEMOLYTICA* SEPTICAEMIA OF LAMBS

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## INTRODUCTION

Descriptions of disease in sheep due to Pasteurellae are common in the literature. A haemolytic Gram-negative cocco-bacillus related to *Pasteurella multocida* and sometimes called *Pasteurella haemolytica* has been described in pneumonias from cattle, calves and most commonly sheep, while related although non-haemolytic strains have also been described in sheep.

Dungal (1931) described outbreaks of acute pneumonia of an apparently infectious nature in sheep in Iceland. The disease frequently resulted in death in less than twelve hours, sheep apparently healthy at night being found dead in the morning. The average duration of the illness was however 24 to 48 hours and chronic cases survived for up to 14 days. At post-mortem examination an extensive consolidation of the lungs was invariably present. From the lungs a Gram-negative, short rod showing bipolar characters in 6-hour cultures was consistently isolated. The organism was non-haemolytic on blood agar. It was occasionally isolated from heart-blood, liver and spleen. Dungal was successful in transmitting the disease to sheep by intratracheal injection of broth cultures. Subcutaneous injection of culture carried out in an attempt at immunisation in a flock of 70 sheep caused lameness and illness in all the animals and a few died.

Montgomerie, Bosworth and Glover (1938) working in Wales, described an enzootic pneumonia in sheep—mostly ewes—often following violent changes in weather or management. Again the course of the disease ranged from 12 hours to 7 days. Lesions were generally confined to the thoracic cavity and consisted of an acute and extensive consolidation of the lungs together with an excess of fibrinous fluid in the pleural cavity but occasionally only extensive congestion of the lungs was present. Of 22 cases examined, four gave negative bacteriological results. In the remainder a pleomorphic, Gram-negative, haemolytic cocco-bacillus was isolated from the lungs only. A distinct capsule was demonstrated. The organism was non-pathogenic to rabbits, guinea-pigs and pigeons but mice succumbed to large doses though this effect was soon lost on sub-culture. The organism proved non-pathogenic for sheep by the intravenous, subcutaneous and conjunctival routes. Tissue inoculations also failed to set up disease. Montgomerie, *et. al.* expressed doubt as to the etiological significance of the haemolytic cocco-bacillus, and considered that the pulmonary invasion of the organism followed the action of a virus or was the result of an environmental change.

Similar organisms have been described from other outbreaks of pneumonia in sheep, e.g. Beveridge (1937) in Australia, Leyshon (1932),

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Spray (1923) and Newsom and Cross (1932) in the U.S.A. Newsom and Cross suggested the name *Pasteurella haemolytica* for the haemolytic form of pasteurella. An organism of the same sub-group has also been isolated from the nasal cavities of normal sheep and cattle (Dungal, 1931; Jones and Little, 1921; Francis, 1941; Bosworth and Lovell, 1944).

With the exception of the pasteurella described by Dungal all the above organisms were non-pathogenic to sheep by experimental inoculation.

Although reports of the association of haemolytic cocco-bacilli with disease in sheep are relatively few in the literature, it is our experience that organisms of this group can be isolated frequently from the lungs of pneumonic sheep in Britain (personal observations and private communications). Their significance has, however, never been easy to interpret. The object of this paper is to report an acute disease of sheep associated with a member of this group, the disease syndrome being of a septicaemic rather than pneumonic character. The organisms isolated were pathogenic to experimental sheep when given intravenously. It is considered that the information presented may help in the future appreciation of the significance of *P. haemolytica* in sheep disease.

## CLINICAL DISEASE

The disease syndrome has been the cause of heavy losses in lambs and hoggs in the Border area of Scotland in recent years, particularly in 1952 and 1953. Its onset usually follows the changing of the hoggs from more permanent pasture to aftermath or foggage in late summer. Commonly no symptoms are noticed, the first indication of disease being the sudden deaths of lambs. In the few cases which have been observed ill, the affected animal tends to separate itself from the flock and if driven readily lies down. Breathing is laboured and slight frothing at the mouth is seen, while the temperature is elevated to 106°F to 107°F. Death in such cases usually occurs within twelve hours of symptoms being seen. The clinical history is very similar to that of pulpy kidney disease or braxy. Once the condition appears, losses are often heavy and 10 per cent. to 20 per cent. of the animals on the affected field may die.

## POST-MORTEM EXAMINATION

The animal is generally in good bodily condition. A blood-stained froth may be present at the mouth or nostrils. On opening the body the muscles are found to be dry but subcutaneous haemorrhages are not usually present.

### *Respiratory System*

The trachea and bronchi are very congested and contain a blood-stained frothy fluid. The lungs are not collapsed, very severely congested and usually heavy with oedema. The pleural surfaces are a slate-blue

colour, while scattered over the sub-pleural surface are numerous darker haemorrhagic areas about 0.5 to 1 cm. in diameter. All lobes have these lesions but the diaphragmatic lobes show the features most clearly. The interlobular septa are very obvious. The cut surface of the lung shows an abundance of a colourless frothy fluid flowing from the squeezed lung. Pneumonic consolidation of the apical and cardiac lobes is only present in those cases which have survived for several days. A clear, blood-tinged pleural fluid is often present in considerable quantity. The mediastinal and bronchial lymph glands are often considerably enlarged, the cut surface being oedematous.

*Cardio-vascular System*

Frequently a large quantity of cloudy fluid containing strands or large clots of fibrin is present in the pericardial sac. The sub-epicardial and endocardial surfaces of the heart often show numerous haemorrhagic splashes. The heart valves may show petechial haemorrhages but no inflammatory thickening.

The spleen is small, firm and poor in blood.

*Alimentary System*

Both the alimentary canal and the liver are abnormal. The abomasum shows either considerable areas of haemorrhagic, exudative inflammation or numerous, shallow, haemorrhagic ulcers which are more numerous at the pyloric end. The lesions are not unlike those described in braxy except that smears of the areas do not show the presence of *Clostridium septique*. Similar lesions may occasionally be found on the folds of the omasum or in the duodenum. Stomach and intestinal worms are not usually numerous and may be absent.

The liver shows some degree of fatty change while congestion of the central veins is considerable but the superficial appearance of the liver in situ would not suggest great abnormality. Careful examination, especially of the cut surface, often shows small (0.5 to 5 mm.) grey or whitish necrotic foci which may be of considerable diagnostic importance. Such lesions can be seen scattered over the cut surface of the liver in varying numbers.

The peritoneal cavity contains a little blood-stained fluid while blood splashes may be present over the visceral peritoneum.

*Urogenital System*

The kidneys are congested and show fatty change, the two together giving a blotchy surface. The cut face shows the cortex to be softer than the medulla. The appearance of the kidneys is similar to that often seen in pulpy kidney disease.

## HISTOLOGICAL EXAMINATION

*Lungs*

Microscopic examination of the lungs shows the venules and capillaries of the alveoli greatly distended and packed with red blood corpuscles while blood extravasations are numerous and vary considerably in size. Pulmonary oedema of the alveoli and of the interlobular septa is present. The amount of fluid varies consider-

ably from case to case and from area to area in any one lung. The oedema fluid may contain fibrin or have a granular appearance but more commonly it stains as a serous fluid. Bacillary emboli in the capillaries of the lung alveoli and of the visceral pleura are numerous. These bacteria distend the capillaries which bulge into the alveolar spaces. There may be little or no tissue reaction to these emboli (Fig. 1) or they may be associated with deposition of fibrin within the vessel. In other cases the emboli may be the centre of a small lesion of necrosis and fibrin deposition (Fig. 2) which is surrounded and infiltrated by an inflammatory reaction. The inflammatory cell types may be polymorphonuclear neutrophils (Figs. 3 and 4) with groups of macrophages plugging the surrounding lung alveoli (Figs. 5 and 6). These lesions are focal in distribution and remain restricted in size so that in the cases described in the present series there is no evidence of bronchial dissemination from the focal lesions. In several cases examined considerable areas of bronchopneumonia were present in the apical and cardiac lobes but the lesions were too advanced to determine whether in fact the pneumonic areas had risen from pulmonary bacterial emboli.

*Liver*

The liver may show some degree of fatty change while congestion may be severe. Bacillary embolism is frequent in both the terminal branches of the interlobular veins and in the liver sinusoids which may be distended with masses of organisms. In a few cases the peripheral liver cells show only slight degeneration but more frequently the surrounding cells undergo considerable degeneration so that focal areas of necrosis arise (Fig. 7). Such lesions may show extensive fibrin deposition in the involved vessels while areas of haemorrhage and leucocytic infiltration may be considerable in the surrounding tissue (Fig. 8). Large numbers of bacilli occur in these degenerating areas both as vascular emboli and also diffusely throughout the degenerated tissue. In a few cases some large interlobular veins contain fibrin clots along with masses of bacteria and one or several associated liver lobules show degenerative and inflammatory changes.

*Spleen*

Numerous bacterial emboli are present in the smaller arterioles but apart from degeneration of the immediately surrounding tissue no other pathological lesions are present.

*Kidney*

A few bacterial emboli may be present in the glomerular arterioles and capillaries but these are not numerous. Emboli are more numerous and occur more frequently in the arterioles of the pelvic area where an inflammatory reaction may be present.

## BACTERIOLOGICAL EXAMINATION

In view of the similarity of the post-mortem lesions to those of entero-toxaemia, routine biological examination for toxins in the intestinal contents was carried out in all cases examined but invariably with negative results. In addition, in 25 per cent. of the cases, a more complete examination was made in that material was taken from various levels of the bowel and treated with trypsin before being inoculated into mice, but again no toxins could be demonstrated. That the disease was not pulpy kidney disease was also supported by the fact that pulpy kidney serum gave no protection in field outbreaks.

Cultures from heart blood, lung, liver, kidney and spleen were made on 10 per cent. bovine blood agar and incubated at 37°C. (no growth occurred at 20°C). In all typical cases examined a characteristic luxuriant growth of a haemolytic organism was present at 15 to 18 hours. Plates incubated anaerobically and in an atmosphere of 10 per cent. $CO_2$ did not give rise to any other organisms though the organism described varied slightly in its growth characteristics under these conditions.

## NATURE OF THE ORGANISM

*Morphology.* The organisms were non-motile, pleomorphic ranging from bipolar cocco-bacilli 0.4$\mu$ x 0.3$\mu$ to rods 2.5$\mu$ x 0.5$\mu$, Gram-negative, and non-sporing. No capsule could be demonstrated by the wet Indian ink method (Duguid, 1951).

*Colony characteristics.* At 18 hours the colony was conical, translucent and slightly fluorescent with an entire edge. At forty-eight hours there was a tendency towards "heaping-up" in the centre which was then much more opaque. The colony surface was smooth and the structure amorphous. Colonies were readily removed by a wire loop and were easily suspended. The growth was similar on serum agar and nutrient agar. An area of clearing was present round the colony on blood. In nutrient broth the organism grew diffusely and rather poorly. A delicate pellicle formed after three days which was readily broken up by movement. In shake culture, colonies occurred throughout the medium but in stab culture the growth tended to be greater at the top. Growth occurred on the needle track alone.

*Biochemical reactions.* The method used was to culture the organisms in peptone water, pH 7.6, to which had been added 1 per cent. of substance under test, the indicator being Andrades. Twenty-four strains were examined. All produced acid but no gas in glucose, saccharose and mannitol. Acid was produced in laevulose, maltose, and sorbitol with the exception of three strains each of which failed to ferment one of these substrates. With one exception, all strains failed to ferment inulin. Reactions with lactose, galactose, mannose, salicin, raffinose, xylose, arabinose, dulcitol, cellobiose, rhamnose, glycerol and dextrin were variable (see Table 1). None of the strains liquefied gelatin or produced indole. The Voges-Proskauer reaction was negative and ammonia was

not produced from peptone after five days' incubation. There was no change in methylene blue milk or litmus milk although slow growth occurred. Organisms were not bile-soluble, did not utilise citrate or hydrolyse urea or starch. No growth was obtained on desoxycholate citrate agar or potato medium but a fair growth was obtained on McConkey's medium with the production of small pink colonies. Nitrates were reduced and the methyl red reaction was negative.

TABLE 1

BIOCHEMICAL REACTIONS

| | *No. of strains* | |
|---|---|---|
| | *Positive (Acid. No gas.)* | *Negative* |
| Glucose | 24 | 0 |
| Laevulose | 23 | 1 |
| Maltose | 23 | 1 |
| Saccharose | 24 | 0 |
| Lactose | 15 | 9 |
| Galactose | 16 | 8 |
| Mannitol | 24 | 0 |
| Mannose | 21 | 3 |
| Salicin | 10 | 14 |
| Inulin | 1 | 23 |
| Raffinose | 13 | 11 |
| Sorbitol | 23 | 1 |
| Xylose | 15 | 9 |
| Arabinose | 16 | 8 |
| Dulcitol | 19 | 5 |
| Cellobiose | 22 | 2 |
| Rhamnose | 16 | 8 |
| Glycerol | 20 | 4 |
| Dextrin | 22 | 2 |
| Inositol | 22 | 2 |
| Liquefaction of gelatin | | — |
| Liquefaction of inspissated serum | | — |
| Growth on McConkey | | + |
| Growth on desoxycholate citrate agar | | — |
| Growth on potato | | — |
| Citrate utilisation | | — |
| Litmus milk | | No change |
| Methylene blue milk | | No change |
| Nitrate reduction | | + |
| Catalase | | + |
| Indole production in peptone water | | — |
| Ammonia production in peptone water | | — |
| Methyl red reaction | | — |
| Voges-Proskauer reaction | | — |
| Bile solubility | | — |
| Starch hydrolysis | | — |
| Cellulose digestion | | — |
| Urea hydrolysis | | — |
| Growth in nutrient broth at pH 5 | | — |
| " " " " " 6 | | — |
| " " " " " 7.6 | | + |
| " " " " " 8 | | + |
| " " " " " 9 | | + |

*Thermal death point.* The organisms withstood exposure for ten minutes but not fifteen minutes to a temperature of 50°C. and for five minutes but not ten minutes to 55°C.

## PATHOGENICITY

Owing to the small number of sheep available it was not possible to carry out detailed pathogenicity tests, but the results of a limited number of transmission experiments are given below.

Tissue and organisms from seven lambs obtained from six field outbreaks of the disease were tested. The organisms from each lamb were not subjected to identical pathogenicity tests but the principal objects of the experiments were : (*a*) to determine if the causal agent of the condition could be transmitted by tissue inoculation with the reproduction of the symptoms and lesions of the natural disease : (*b*) to determine whether the *P. haemolytica* alone could produce the symptoms and lesions.

### *Materials and Methods*

The materials used for transmission purposes were lung suspensions, plate washings and serum, and nutrient broth cultures.

*Lung suspension.* Five grammes of lung were ground in a Griffith's tube with 10 ml. nutrient broth, the resultant pulpy mixture being lightly centrifuged to throw down particulate matter. The cloudy, red supernatant fluid was used as the inoculum.

*Plate washing.* This preparation was made from original culture plates on 10 per cent. ox blood agar. The culture used was generally from the lung as this tissue consistently yielded the richest growth of the organism. After 24 hours' incubation the plate was flooded with 5 ml. nutrient broth and the colonies broken from the solid medium by gentle rubbing with a glass rod. The resultant rather granular suspension contained an average of $15 \times 10^8$ organisms per ml.

*Nutrient and serum broth cultures.* Nutrient broth and broth enriched with 5 per cent. ox serum were inoculated from the original plates and incubated for eighteen hours. The addition of serum did not make any appreciable difference, the numbers of organisms present at 18 hours being consistently $10^{8.5}$ per ml.

### *Initial Transmission Experiments (Table 2)*

*Case* 1087/3. A lung suspension in a dose of 2 ml. given intravenously and subcutaneously killed experimental hoggs in 12 hours with clinical symptoms and a post-mortem picture typical of the natural disease. Similar results were obtained using a spleen pulp suspension prepared in the same way as the lung. The washings from the original plate culture (3 ml. dose) given intravenously resulted in death in 12 hours and a typical post-mortem picture. Three ml. of a broth culture given subcutaneously resulted in an acute purulent cellulitis, the experimental hogg having to be destroyed in 48 hours. In the cases where death resulted, *P. haemolytica* was isolated in pure culture from all organs and

from the blood. In the cellulitis case a rich growth of the organism was obtained from the serous purulent exudate and a few bacterial colonies were cultured from the spleen.

Lung suspension, prepared from the experimental hogg which died following intravenous injection of lung pulp from the field case, was further passaged by the intravenous route and resulted in a death and a typical post-mortem picture.

*Case* 1063. A lung suspension was administered by the intravenous, intratracheal and subcutaneous routes respectively. The intratracheal dose was 10 ml., the intravenous and subcutaneous doses being 2 ml. None of these animals showed any apparent ill effects.

*Case* 1071. Original plate washings in doses of 2 ml. were administered by the intratracheal and subcutaneous routes respectively. The hogg receiving the intratracheal inoculation remained healthy. An acute cellulitis developed in the sheep inoculated subcutaneously. Recovery in the latter case was slow and took over fourteen days.

*Case* 1127/1. The original plate washing was given intravenously in a 2 ml. dose. The animal suffered no apparent ill effects.

*Case* 1146. The original plate washing was given intravenously in a 2 ml. dose. Death with a typical post-mortem picture occurred in 12 hours. The organism was recovered from the organs and heart blood.

*Case* 1190. The original plate washing given intravenously in a 2 ml. dose resulted in death with a typical post-mortem picture in 12 hours. *P. haemolytica* was cultured from all the organs and heart blood.

*Case* 1118/2. A lung suspension in doses of 2 ml. was administered intravenously, intratracheally and subcutaneously. The intravenous injection caused death with a typical post-mortem picture, *P. haemolytica* being recovered from the organs and heart blood. No ill effects were suffered by the animals receiving the intratracheal and subcutaneous injections.

A spleen suspension given subcutaneously in a 2 ml. dose resulted in an acute cellulitis necessitating the destruction of the animal in 3 days. *P. haemolytica* was recovered from the affected tissues.

An original plate washing was administered subcutaneously and intravenously in 2 ml. doses. Death with the typical post-mortem picture and recovery of the organism occurred after the intravenous inoculation. The subcutaneous injection resulted in an acute cellulitis with oedema, lameness and fever. The animal made a slow recovery over three weeks.

*Second Series of Transmission Experiments*

*Case* 1118/2. Lung tissue which had been stored at —60°F was inoculated on to blood agar. The resultant, apparently pure, cultures of *P. haemolytica* were washed off a plate and 4 ml. were inoculated intravenously into a sheep. The animal died with the typical post-mortem picture and bacteriological findings.

As the pathogenicity of the organism had not declined on storage, the material was used to attempt to find the average lethal dose. Accurate

TABLE 2

INITIAL TRANSMISSION EXPERIMENTS

| *Case* | *Material* | *Intra-venous* | *Sub-cutaneous* | *Intra-tracheal* (10 ml.) | *Dose* | *Remarks* |
|---|---|---|---|---|---|---|
| 1087/3 | L.S. | + | + | 0 | 2 ml. | |
| 1087/3 | S.S. | + | + | 0 | 2 ml. | |
| 1087/3 | P.W. | + | 0 | 0 | 3 ml. | |
| 1087/3 | B.C. | 0 | Cellulitis | 0 | 3 ml. | |
| *1087/3A | L.S. | + | 0 | 0 | 2 ml. | *This material was from hogg dying after intravenous L.S. 1087/3. |
| *1087/2A | B.C. | + | 0 | 0 | 4 ml. | *This strain was recovered after one guinea-pig transmission of organism from 1087/2. |
| 1063 | L.S. | — | — | — | 2 ml. | |
| 1071 | P.W. | 0 | Cellulitis | — | 2 ml. | |
| 1127/1 | P.W. | — | 0 | 0 | 2 ml. | |
| 1146 | P.W. | + | 0 | 0 | 2 ml. | |
| 1190 | P.W. | + | 0 | 0 | 2 ml. | |
| 1118/2 | L.S. | + | — | — | 2 ml. | |
| 1118/2 | S.S. | 0 | Cellulitis | 0 | 2 ml. | |
| 1118/2 | P.W. | + | Cellulitis | 0 | 2 ml. | |

+ = Death in 12 hours
— = No effect
0 = Not done

L.S. = Lung emulsion
S.S. = Spleen emulsion
B.C. = Broth culture
P.W. = Plate washing

results were again hindered by shortage of experimental animals and only one animal was available for each test dose. Doses of 6, 4, 2 and 1 ml. respectively were administered intravenously. A polyarthritis developed within two days in the animal receiving the 2 ml. dose and a blood sample taken at 24 hours showed an acute bacteraemia. The animal died twelve days later and at post-mortem examination the joints contained a muco-purulent exudate from which no bacteria could be isolated. The sheep receiving 6, 4 and 1 ml. doses respectively died within 12 hours and showed the usual post-mortem lesions. The sheep receiving 4 ml. had, in addition to the usual lesions, a considerable quantity of clear pleural exudate from which *P. haemolytica* was grown in considerable numbers.

A plate washing from the above pleural fluid, which had been freeze-dried, was given intravenously to two sheep in doses of 4 ml. and 6 ml. respectively. Both sheep died and *P. haemolytica* was recovered from all the organs. At the same time colonies from the blood agar plates were inoculated into 10 ml. of nutrient and serum broth. After 24 hours' incubation 6 ml. of each were given intravenously to two sheep. The one which received the serum broth died within 12 hours with the usual post-mortem findings. The other inoculated with the nutrient broth developed a persistent polyarthritis and was destroyed three weeks later when cultures from the muco-purulent joint fluid gave negative results. However the inoculation of 12 ml. of the 24-hour nutrient broth culture into another sheep resulted in death in twelve hours. The inoculation of two more sheep using 6 ml. doses of nutrient broth and serum broth cultures this time caused polyarthritis in both sheep. The sheep which received the nutrient broth culture was destroyed at 2 days but that receiving the serum broth culture made a slow recovery.

*Pathology of the Disease Set up by Experimental Inoculation*

The post-mortem appearances of sheep killed by experimental inoculation were similar to those described in the natural disease and especially obvious werethe haemorrhagic and ulcerative areas of the abomasum. Histological examination showed similar lesions to those already described except that in four cases the number of bacterial emboli in the liver, lung and spleen were very few. In the majority of cases, however, including those where death occurred after subcutaneous inoculation, lesions identical with those described in the natural disease were present. The general impression was, however, that, although the bacterial emboli were as numerous as in the natural cases the surrounding inflammatory reaction and tissue destruction were on the whole not so extensive as in the lesions of the natural disease. If death did not occur after subcutaneous inoculation, widespread, severe and purulent cellulitis invariably occurred. In a number of cases where death did not follow intravenous inoculation a severe purulent polyarthritis arose as a result of the inoculation and destruction of the animal had to be carried out on humane grounds.

## DISCUSSION

It has been recognised for a considerable time that *P. haemolytica* can be isolated from pneumonic lungs of sheep but there has been considerable doubt whether this organism is in fact capable of causing the disease. The view is now commonly held that a filterable virus may be the initiating factor (Dungal, 1931; Newsom and Cross, 1932). The National Veterinary Medical Association handbook on sheep diseases (1949) classifies the pneumonias as being of unknown etiology and suggests that a virus has been suspected but it has not been demonstrated. It is also stated that a pasteurella-like organism is consistently isolated from affected sheep but that under normal conditions this organism may be saprophytic but becomes pathogenic either by a lowering of the natural resistance of the sheep or by an unexplained increase in its own virulence. Greig in *The Shepherd's Guide* (1951) states that the pasteurella organism is a normal inhabitant of the respiratory tract of healthy sheep, but that the factors which cause it to become actively virulent are obscure. He states that it is possible that the main activating agent is an infective virus.

That *P. haemolytica* has been assigned a secondary rôle in sheep diseases is possibly due to the fact that its pathogenicity to the experimental animal has been reported in the literature as being low whereas *Pasteurella septica* is reported as being pathogenic both to laboratory and domestic animals on experimental inoculation. It would also appear from the literature that the lesions described as being associated with *P. haemolytica* are generally confined to the

thoracic cavity. Hagan and Bruner (1951) state that if an acute, bacteraemic form of pasteurella infection occurs in cattle and sheep it is apparently very rare in the United States. Gaiger and Davies (1946) also suggest that in Britain the septicaemic form of pasteurellosis is rare and that the majority of the outbreaks are forms of enzootic pneumonia and that the lesions are confined to the thoracic cavity.

In the South East of Scotland for a number of years an acute and fatal disease of lambs commonly called "pneumonia" has been common in the autumn. A method of prevention against this disease carried out by a large number of farmers is the vaccination of the lambs with pasteurella vaccines, with very indifferent results. Over the last several years, a few of a considerable number of such lambs which have been examined post-mortem have shown extensive lesions of bronchopneumonia in the apical and cardiac lobes from which *P. haemolytica* was isolated, but many have not. Considerable doubt has been expressed by us whether the diagnosis of pneumonia in such cases was justified and the opinion based on post-mortem appearances of the lambs was that the deaths might in fact be caused by *Clostridium welchii* type D toxins. Unfortunately, in the majority of cases such toxins could not be demonstrated but since the usual advice to change the grazing to a poorer type was generally successful in stopping deaths from occurring, it was considered that the methods used to demonstrate the toxins of *Cl. welchii* were not adequate. During the autumn of 1953 the number of deaths caused by "pneumonia" were more numerous than usual and, as it was not possible to arrest losses in the field by the use of pulpy kidney serum, it was concluded that some other factor than *Cl. welchii* toxin was the cause of death. Since *P. haemolytica* was cultured in considerable numbers from lung, spleen, heart blood, kidney, etc., and the histological lesions suggested that the organisms were perhaps not so harmless as had been assumed, it was decided to carry out preliminary pathogenicity experiments using lambs 7 to 9 months old. The results show that death in 12 hours with characteristic post-mortem, bacteriological and histological findings may follow intravenous inoculation of 1 ml. of lung or spleen suspension and that serial passage gives similar results. A lung suspension from a normal sheep is non-pathogenic. Lambs inoculated intravenously with 2 ml. of a broth washing of a primary plate culture of *P. haemolytica* also died in 12 hours with the characteristic post-mortem and bacteriological findings. Subcutaneous inoculation of similar material resulted in either death within 12 hours or in an extensive cellulitis at the site of inoculation. Intratracheal injection was not a successful route for infection purposes.

Tissue from both field and experimental sheep as well as freeze-dried cultures stored for several months in the deep-freeze at —60°F. retained their pathogenicity.

An attempt to find a standard lethal dose by the inoculation of lambs with broth washings of primary plate cultures of *P. haemolytica* by the intravenous route showed that the lethal dose varied from 1 ml. to 6 ml. If death did not follow the inoculation in 12 to 18 hours a severe polyarthritis occurred which might be followed by death in a number of days but, on the other hand, the animal might slowly recover. So far as the investigation has gone the attempt to find a standard lethal dose suggests that the resistance of sheep varies considerably though the numbers of animals used is too small for definite conclusions. There is also some evidence that young sheep are more susceptible than old sheep but again this requires further investigation. This varying susceptibility of the experimental animal may also be the explanation for the apparent lack of virulence of the organism isolated from the field case 1127/1 where 2 ml. of a plate washing failed to have any effect on the inoculated sheep. It is unfortunate that the cost of the sheep as an experimental animal limited the number of animals which could be used but it is considered that despite this limitation the evidence is still convincing that the present strains of *P. haemolytica* are pathogenic for sheep.

It was considered possible that the plate washing from a primary blood agar culture might be carrying over some factor other than the pasteurella organism but the experimental results do not support this suggestion and it would in fact appear that the evidence presented fulfils "Koch's postulates" as to the causal relationship of *P. haemolytica* to the disease found in the field.

Although *P. haemolytica* is the specific cause of the pathological changes there are undoubtedly other factors in the etiology of the disease. The epidemiology of the disease in the field suggests that environmental factors are probably more important than epidemic strains of organisms since outbreaks of the disease can be halted almost immediately by changing the sheep's grazing. This is not unusual in some other sheep diseases since similar etiological factors seem to play a part in the entero-toxaemias caused by *Cl. welchii* type D, in braxy caused by *Cl. septique* and in blackquarter.

It has generally been assumed that *P. haemolytica* invades the tissues of the body from the respiratory tract, perhaps secondary to a virus infection. The histological lesions in the field outbreaks described in the paper are those of a septicaemia and there is nothing to suggest that the primary site of invasion was the lung. On the contrary, in several cases where some pneumonic lesions were present it is considered that these were secondary to bacterial thrombi in the pulmonary vessels. In the present investigation it was not possible to determine the primary site of invasion of the organism although the number of *P. haemolytica* organisms present in the mesenteric lymph glands might suggest that it took place from the alimentary canal.

It has been suggested that *P. haemolytica* is frequently present in the naso-pharynx of healthy sheep. There is no doubt that such organisms have the general characteristics and biochemical reactions of the group but since these strains have not been properly investigated there must be considerable doubt whether they are in fact identical with the *P. haemolytica* isolated from enzootic pneumonia and from the disease at present being described. Until more work has been carried out it would be unwise to suggest that such commensal organisms play any part in the epidemiology of these diseases of sheep.

The similarity of the post-mortem findings in the present investigation to those of braxy must also be considered especially as gastric ulceration and haemorrhage were usually present. None of the lambs which were examined had been vaccinated against braxy but we were unable to demonstrate *Cl. septique* in any appreciable numbers in the lesions of the stomach.

## CONCLUSIONS

The pathological and bacteriological findings in lambs dead from *P. haemolytica* septicaemia have been investigated.

A description is given of the cultural characteristics of the organisms isolated.

Pathogenicity tests using the sheep as the experimental animal were carried out.

## REFERENCES

Beveridge, W. I. B. (1937). *Aust. vet. J.*, **13**, 155.
Bosworth, T. J., and Lovell, R. (1944). *J. comp. Path.*, **54**, 168.
Duguid, J. P. (1951). *J. Path. Bact.*, **63**, 673.
Dungal, N. (1931). *J. comp. Path.*, **44**, 126.
Francis, J. (1941). *Brit. vet. J.*, **97**, 243.
Gaiger, S. H., and Davies, G. O. (1946). *Veterinary Pathology and Bacteriology*. Baillière, Tindall and Cox; London.
Greig, J. R. (1951). H.M. Stat. Office; Edinburgh.
Hagan, W. A., and Bruner, D. W. (1951). *Infectious Diseases of Domestic Animals*. Baillière, Tindall and Cox; London.
Jones, F. S., and Little, R. B. (1921). *J. exp. Med.*, **34**, 541.
Leyshon, W. J. (1932). *Vet. J.*, **88**, 256.
Montgomerie, R. F., Bosworth, T. J., and Glover, R. E. (1938). *J. comp. Path.*, **51**, 87.
Newsom, I. E., and Cross, F. (1932). *J. Amer. vet. med. Ass.*, **80**, 711.
—— (1949). *Report on Diseases of Farm Livestock, Diseases of Sheep*. Nat. vet. med. Assoc. Gt. Britain.
Spray, R. S. (1923). *J. inf. Dis.*, **33**, 97.

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Fig. 1. Lung showing bacterial emboli, pulmonary oedema and congestion. The oedema fluid may contain fibrin but more commonly it stains as a serous fluid. Giemsa × 200

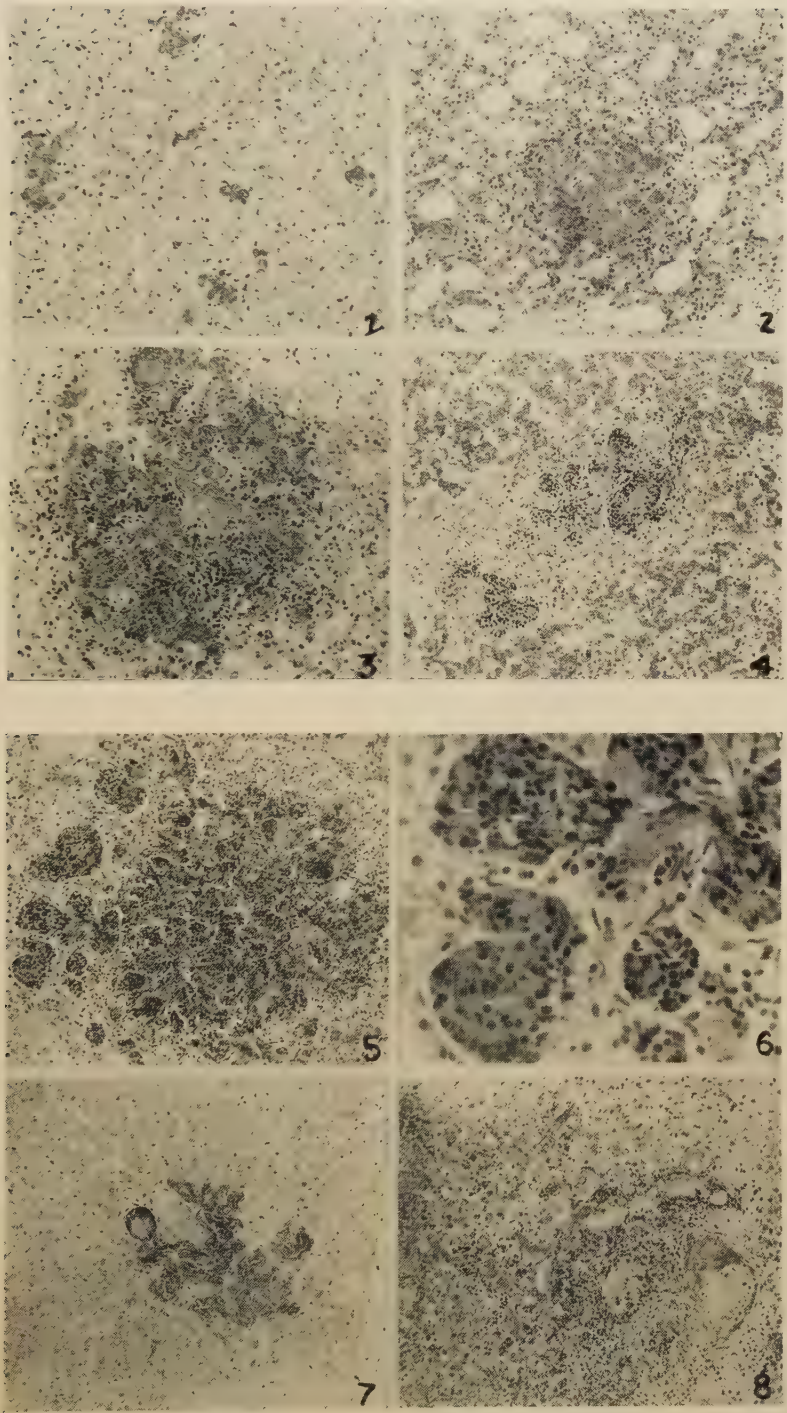
Fig. 2. Lung showing bacterial embolus associated with a focal lesion of necrosis and fibrin deposition. Giemsa × 500

Figs. 3 & 4. Lung showing embolus surrounded by an inflammatory reaction, the cell type being a polymorphonuclear neutrophil. Fig. 3 H. and E. × 200
Fig. 4 H. and E. × 150

Figs. 5 & 6. More advanced lesion than in figs. 3 and 4, the lung alveoli being plugged with groups of macrophages. Fig. 5 H. and E. × 150
Fig. 6 H and E. × 500

Fig. 7. Liver showing bacillary embolism and associated necrosis of liver cells. Giemsa × 150

Fig. 8. Liver lesions showing necrosis, extensive fibrin deposition and inflammatory cells. H. and E. × 150




*To face page* 196

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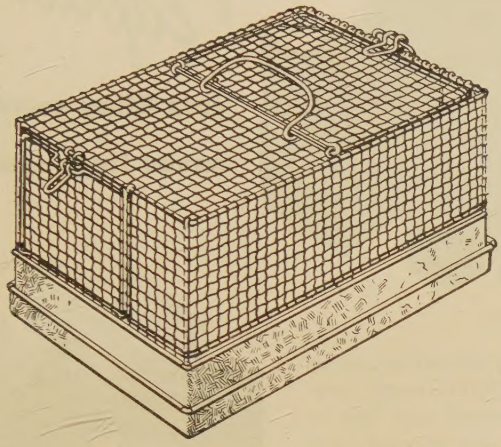

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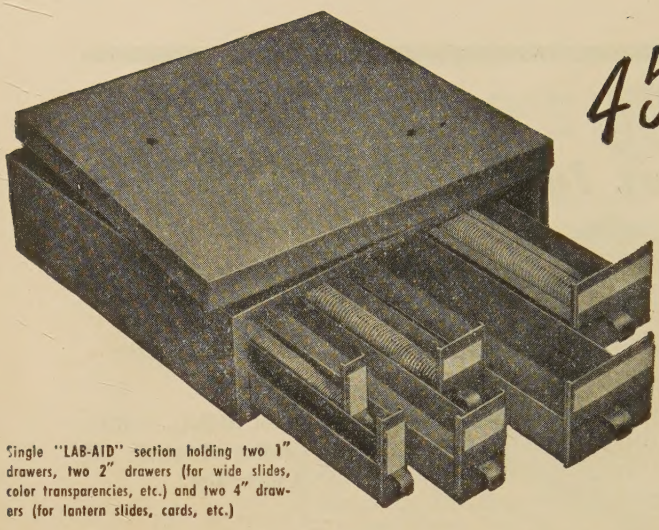

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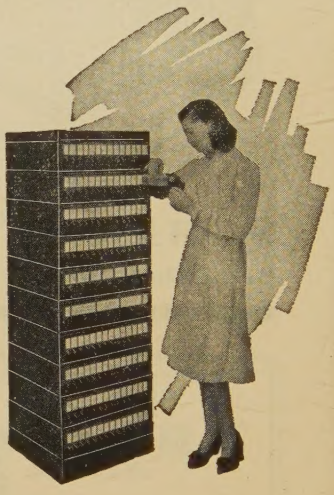

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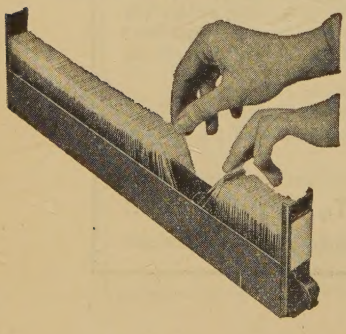

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